METHOD DEVELOPMENT AND VALIDATION OF SULTAMCILLIN TOSYLATE IN TABLET DOSAGE FORM BY RP-HPLC AND HPTLC

DISSERTATION

Submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY,
CHENNAI.

In partial fulfilment for the award of the degree of

MASTER OF PHARMACY
In
(Pharmaceutical Analysis)

By

26101134

Under the Guidance of

Dr.SHANTHA ARCOT, B.pharmM.Sc (Pharm).,Ph.D. .,

Head of Department, Department of Pharmaceutical Analysis.

C.L.BAID METHA COLLEGE OF PHARMACY

CHENNAI – 600 097.

SEPTEMBER– 2012

Phone : 24960151, 24960425
E-mail : principal@clbaidmethacollege.com
Website : www.clbaidmethacollege.org

C.L. Baid Metha College of Pharmacy

An ISO 9001 - 2000 certified institution

Jyothi Nagar, Old Mahabalipuram Road
Thorapakkam, Chennai - 600 097.

Affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education. New Delhi.

SRI. VINOD KHANNA
Chairman

SRI UDAY.L.METHA
Secretary &Correspondent

Dr.SHANTHA ARCOT,B.pharmM.Sc. Pharm, Ph.D.
HOD, Department of Pharmaceutical analysis

Dr. GRACE RATHNAM, M.Pharm.,Ph.D.
Principal

CERTIFICATE

This is to certify that the project entitled**"METHOD DEVELOPMENT AND VALIDATION OF SULTAMCILLIN TOSYLATE IN TABLET DOSAGE FORMBY RP-HPLC AND HPTLC "by 26101134**submitted in partial fulfilment for the award of degree of **Master of Pharmacy** and itwas carried out at aurobindo pharmaceuticals, vishakapatnam under the supervision of **Mr. srinivas M.Pharm.,** R&D Manager **and Mrs. Navatha B.Pharm.,**R&D Assistant manager**,** and at C.L.Baid Metha college of Pharmacy, Chennai-96 Under the supervision of **Mrs. A. Shantha,B.Pharm M.Sc.(Pharm)., Ph.D.,** Head of the department, during the academic year 2011-2012.

Date:

Dr .A. Shantha,B.pharmM.Sc.(Pharm).,Ph.D.
HOD, Department of Pharmaceutical Analysis
C.L.Baid Metha College of Pharmacy,
Chennai-96.

Phone : 24960151, 24960425
E-mail : principal@clbaidmethacollege.com
Website : www.clbaidmethacollege.org

C.L. Baid Metha College of Pharmacy

An ISO 9001 - 2000 certified institution

Jyothi Nagar, Old Mahabalipuram Road
Thorapakkam, Chennai - 600 097.

Affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education. New Delhi.

SRI. VINOD KHANNA
Chairman

SRI UDAY.L.METHA
Secretary & Correspondent

Dr.SHANTHA ARCOT, B.PharmM.Sc.Pharm, Ph.D
HOD, Department of Pharmaceutical analysis

Dr. GRACE RATHNAM, M.pharm., Ph.D
Principal

CERTIFICATE

This is to certify that the project entitled **"METHOD DEVELOPMENT AND VALIDATION OF SULTAMCILLIN TOSYLATE IN TABLET DOSAGE FORM BY RP-HPLC AND HPTLC" by 26101134** submitted in partial fulfilment for the award of degree of **Master of Pharmacy** and itwas carried out at Aurobindo pharmaceuticals Vishakhapatnam under the supervision of **Mr. srinivas, M.Pharm.,** R&D Manager **and Mrs. navatha, B.Pharm.,**R&D Assistant manager**,** andat C.L.Baid Metha college of Pharmacy, Chennai-96 during the academic year 2011-2012. Under the supervision of **Mrs. A. Shantha, M.Sc.(Pharm)., Ph.D.,** Head of the department, Department of Pharmaceutical Analysis, **during the academic year 2011-2012.**

Date :

Dr. GRACE RATHNAM,M.pharm.,Ph.D
Principal
HOD, Department of Pharmaceutics,
C.L.Baid Metha College of Pharmacy,
Chennai-96.

DECLARATION

The thesis entitled**"METHOD DEVELOPMENT AND VALIDATION OF SULTAMCILLIN TOSYLATE IN TABLET DOSAGE FORMBY RP-HPLC AND HPTLC"**was carriedout by me in Department of Pharmaceutical Analysis, C.L.Baid Metha Collegeof Pharmacy, and Chennai – 96 during the academic year 2011-2012. The work embodied in this thesis is original, and is not submitted in part or full for any other degree of this or any other University.

Date: **[Reg.No:26101134]**

Place: Chennai DEPT. OF PHARMACEUTICAL ANALYSIS

ACKNOWLEDGEMENT

Behind every achievement and success of this project work lies an unfathomable sea of gratitude to those who activated it, without whom it would ever have come in to existence to them we lay the words of gratitude imprinted with in us.

I consider myself lucky to work under the guidance of Dr**. Shantha Arcot, M.Sc. (Pharm)**.,**Ph.D**, Head of the department, Department of Pharmaceutical Analysis for her valuable suggestions and personal encouragement were of great importance to improve the outcome of the project.

It's my privilege to express my grateful and sincere gratitude to **Dr.Grace Rathnam, M.Pharm., Ph.D,**principal, C.L.Baid Metha College of Pharmacy.

I am deeply indebted to my respective and dynamic guide **Mrs.Vijayageetha, M.Pharm., (Ph.D)** Assistant professor**,** Department of Pharmaceutical **Analysis**, C.L.Baid Metha College of Pharmacy, Chennai – 96, for her supportand valuable suggestion during my project work.

I acknowledge my sincere thanks to **Mrs. Vijaya Nagarajan, M.Pharm (PhD)** Assistant professor**, Mrs.Uma, M.Pharm,** Assistant professor**, Mrs.Yamuna M.Pharm (PhD)** Assistant professor,Department of pharmaceutical Analysis and all other **teaching staff** of our college for their valuable suggestions through out my thesis work.

I would like to use this opportunity to thank **Mr. Srinivas, M.pharm, Manager and Ms. Navatha B.Pharm, Aurobindo pharmaceuticals** for their kind cooperation rendered in fulfilling my work.I owe my hearty thanks to **Mr. Pandae, HR department, Aurobindo pharmaceuticals**helped to complete my project at time.

I extremely indebted to **Mrs. Rama,M.Pharm. Assistant professor, C.L.Baid Metha College of pharmacy, and Mr.Manikandan, M.Pharm,Assistant professor, SRM University**helped to complete my project at a right time.

I specially thank Ms.**G.Surya,MSc, In charge of ceal lab** for her guidance and source of inspiration that I have received from her throughout my project work. Her continuous encouragement and support have always propelled me to perform better. I am and I would remain grateful to her.

I extend my sincere thanks to Librarian **M. Raja Lakshmi**C.L.Baid Metha College of Pharmacy in helping me to utilize the library facilities for references.I thank all non-teaching staff members of C.L.Baid Metha College including**Mrs.R.Usha, Mrs.Valli** and **Mrs.A.P.Kalpakam** for their help extended during my project work. I specially thank my mother for her support. My humble thanks to almighty, who gave me strength and confidence all the long.

Date:

Reg.No :26101134

DEPT.OFPHARMACEUTICAL ANALYSIS

CONTENTS

LIST OF ABBREVIATIONS

CAN	Acetonitrile
Atm	Atmosphere
Cm	Centimetre
Conc.	Concentration
C GMP	Good manufacturing practices
C GLP	Good laboratory practices
0 c	Degree centigrade
Fig	Figure
Gm	Grams
HPLC	High performance liquid chromatography
HPTLC	High performance thin layer chromatography
ICH	International Conference on Harmonisation
i.d	Internal diameter
LC	Liquid chromatography
LOD	Limit of detection
LOQ	Limit of quantification
Mg	Milligrams
Min	Minutes
Ml	Millilitre
mM	Millimolar
Mm	Millimetre
Mw	Molecular weight
Mg	micro gram
Ml	micro litre
Mm	micro metre
Nm	Nanometre
NLT	Not Less than
NPLC	Normal phase liquid chromatography
NMT	Not more than

pH	Negative logarithm of hydrogen ion
pKa	Dissociation constant
PA	Peak Area
Pa	Pascal
%	Percentage
RI	Refractive index
RP-HPLC	Reverse phase high performance liquid chromatography
RSD	Relative standard deviation
RT	Retention time
SD	Standard deviation
SEC	Size exclusion chromatography
Std	Standard
TLC	Thin layer chromatography
UV-VIS	Ultra-violet-visible
USP	United states pharmacopeia
Vs	Versus
v/v	volume/volume

LIST OF TABLES IN HPLC

LIST OF TABLES USED IN HPTLC

LIST OF CHROMATOGRAMS FOR HPLC

LIST OF CHROMATOGRAMS FOR HPTLC

Introduction

INTRODUCTION

Pharmaceutical Analysis[1,2]is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and quantitative measurements of the substances present in bulk and pharmaceutical preparation.

Pharmaceutical analysis derives its principles from various branches of science like Chemistry, Physics, Microbiology, Nuclear Science, Electronics, etc. Analytical method is a specific application of a technique to solve an analytical problem. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and the environment. This instrumentation provides the lower detection limits required to assure safe foods, drugs, water and air.

Analytical chemistry[3]deals with methods for determining the chemical composition of samples of matter. A qualitative method yields information about the identity of atomic or molecular species or the functional groups in the sample. A quantitative method, in contrast, provides numerical information as to the relative amount of one or more of these components.

TYPES OF ANALYSIS[4]

Proximate analysis- The amount of each element in a sample is determined with no concern as to the actual compound present

Partial analysis- The selected constituent in a sample is determined by partial analysis

Trace constituent analysis- The very minute quantity of a specified component in a sample is determined by this method.

Complete analysis Based upon

1)Quantitative chemical analysis based on chemical reactions

Gravimetry

Titrimetry

Volumetry

2) Appropriate electrical measurements

3) Mechanical properties

4) Thermal properties

5) Nuclear properties

6) Properties involving interaction with radiation

7) Chromatography

8) Hyphenated techniques

CHROMATOGRAPHY

Definition

Chromatography is a non-destructive procedure for resolving a complex mixture into its individual fractions (or) compounds.

Principle

The samples are subjected to flow by mobile liquid phase onto (or) through the stable stationary phase. As in the definition, the principle involved is separation of fractions of mixture based on their relative affinity towards the two phases during their travel. The fraction with greater affinity to stationary phase travels slower. While that with less affinity travels faster.

TABLE:-1[5]

Technique	Stationary phase	Mobile phase
Column chromatography	Solid	Liquid
Partition chromatography	Liquid	Liquid
Paper chromatography	Liquid	Liquid
Thin layer chromatography	Liquid or solid	Liquid
Gas liquid chromatography	Liquid	Gas
Gas solid chromatography	Solid	Gas
Ion exchange chromatography	Solid	Liquid

TYPES OF CHROMATOGRAPHY[6,1]

Based on the **mode** employed in separation chromatography is broadly classified as

1.Adsorption mode

The stationary phase is a solid while the mobile phase is liquid. The compounds travel on the stationary phase under the influence of mobile phase based on their relative adsorption to the solid stationary phase.

2. Partition mode

In this mode, both the stationary and mobile phase was liquids. So the compounds have affinity based on their partition into the individual liquid phases. The one with greater partition to stationary phase has higher affinity to stationary phase and vice versa.

Based on the **nature of stationary phase,** it is of two types

A) Normal phase chromatography

Here the stationary phase is polar in nature and hence the compounds with higher polarity elute out last while non-polar come out first.

B) Reverse phase chromatography

Here the stationary phase is non-polar in nature and hence the compounds with lower polarity elute out last and vice-versa.

Chromatography methods can be broadly divided into planar chromatography and columnar chromatographic methods.

In planar chromatography, the stationary phase is a plane surface i.e. (two dimension surface where only length and breadth are taken as area) on which chromatograms are formed.

This method is adopted in techniques like:-

1. Paper chromatography[5]This technique is a type of partition chromatography in which the substances are distributed between two liquids i.e one is the stationary liquid(usually water) which is held in the fibers of the paper and called the stationary phase. The other is the moving liquid or developing solvent and called the moving phase. The components of the mixture to be separated migrate at different rates and appear as spots at different points on the paper.

2. Thin layer chromatography[4]It is similar to paper chromatography except that a thin (0.25mm) layer of some inert material. The important difference between TLCand HPLC is one of practical technique rather than of the physical phenomena(adsorption, partition, etc.) on which separation is based. Thus in TLC the stationary phase consists of a thin layer of sorbent (e.g. silica gel or cellulose powder) coated on an inert, rigid, backing material such as a glass plate or plastic foil so that the separation process occurs on a flat essentially two-dimensional surface. Although TLC is widely used for qualitative analysis, it does not in general provide quantitative information of high precision and accuracy. Yasa4

4. Gas-liquid chromatography[3] The mobile phase is a gas and the stationary phase is a thin layer of a non-volatile liquid bound to a solid support. Partition process occurs.

5. High performance thin layer chromatography (HPTLC):-

In Columnar chromatography, there is use of a column on whose walls lies a stationary phase and the mobile phase is flushed through the column.

The techniques which employ this method are:-

1. Column chromatography[5]Also known as adsorption chromatography. In this method, the mixture to be separated is dissolved in a suitable solvent and allowed to pass through a tube containing the adsorbent. The component which has greater absorbing power is adsorbed in the upper portion of the column. The next component is adsorbed in the lower portion of the column which has lesser adsorbing power than the first component. This process is continued. As a result the materials are partially separated and adsorbed in the various parts of the column. The initial separation of the various components can be

improved by passing either the original or some other suitable solvent slowly through the columns. The various bands present in the column become more defined. The banded column of adsorbent is termed a chromatogram, and the operation is spoken of as the developmentof chromatogram . the portion of a column which is occupied by a particular substance is called its zone. The narrower the zones, the longer the number of substances which can be separated in a column of a definite length, and the more concentrated are the elutes.

2. Gas-solid chromatography[3]It utilizes a solid adsorbent as the stationary phase, and an adsorption process takes place.

3. High Pressure liquid chromatography (HPLC)[7]In this system pressure is applied to the column, forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution.

4. Size exclusion chromatography[1]It uses a porous material as the stationary phase and a liquid as a mobile phase. The diameters of the pores of the material are of the order of 50-3000A, Which is similar to the size of many molecules. The latter penetrate the pores according to their size. Small molecules penetrate more rapidly than larger molecules, which frequently are excluded from the smaller pores present. This results in a difference in the rates at which the molecules pass down the column, the smaller molecules travelling faster than the larger molecules.

5 .Ion-exchange chromatography[1]Ion exchange chromatography is the process based on exchange equilibria between ions in solution and ions of like sign on the surface of an essentially insoluble high molecular mass solid . Natural ion exchangers are clay and zeolite. Synthetic resins are : strong acid cation exchangers; weak acid cation exchangers; strong base anion exchangers; and weak base anion exchangers.

Analytical method development [8]

The number of drugs introduced into the market is increasing every year. These drugs may be either new entities or partial structural modification of the existing one. Very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, reports of new toxicities (resulting in their withdrawal from the market), development of patient resistance and introduction of better drugs by competitors. Under these conditions, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.

Basic criteria for new method development of drug analysis

The drug may not be official in any pharmacopoeias,

A proper analytical procedure for the drug may not be available in the literature due to patent regulations,

Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients,

Analytical methods for the quantitation of the drug in biological fluids may not be available,

Analytical methods for a drug in combination with other drugs may not be available,

The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY[9]

In 1903, Mikhail Tswett discovered Chromatography technique. It serves as a means of resolution of mixtures. The name suggests Chroma meaning “colour and graphein means “write”.

HPLC is one of the types of Chromatography. In modern pharmaceutical industries, HPLC is the major and integral analytical tool applied in all stages of drug discovery, development, and production. Effective and fast method development is of paramount importance throughout this drug development life cycle. This requiresathorough understanding of HPLC principles and theory which lay a solid foundation for appreciating the many variables that are optimized during fast and effective HPLC method development and optimization.

HPLC as compared with the classical LC technique is characterized by
high resolution[12]
small diameter (4.6 mm), stainless steel, glass or titanium columns;
column packing with very small (3, 5 and 10 μm) particles;
relatively high inlet pressures and controlled flow of the mobile phase;
continuous flow detectors capable of handling small flow rates and detecting very small amounts
rapid analysis;

Initially, pressure was selected as the principal criterion of modern liquid chromatography and thus the name was "high pressure liquid chromatography" or HPLC. This was, however, an unfortunate term because it seems to indicate that the improved performance is primarily due to the high pressure. This is, however, not true. In fact, high performance is the result of many factors: very small particles of narrow distribution range and uniform pore size and distribution, high pressure column slurry packing techniques, accurate low volume sample injectors, sensitive low volume detectors and, of course, good pumping systems. Naturally, pressure is needed to permit a given flow rate of the mobile phase.

HPLC separations are based on the surface interactions, and depend on the types of the adsorption sites. Modern HPLC adsorbents are the small rigid porous particles with high surfacearea.

Main adsorbent parameters ar**e**[13]

Particle size: 3 to 10 μm

Particle size distribution: as narrow as possible, usually within 10% of the mean;

The last parameter in the list represents an adsorbent surface chemistry.

Depending on the type of the ligand attached to the surface, the adsorbent could be normal phase (-OH, -NH2), or reversed-phase (C5, C8, C 18 CN, NH2), and even anion (CH2NR3+OH-), or cation (R-SO3-H+) exchangers.

Principle[10]

The principle of separation is normal phase mode and reverse phase mode is adsorption. When mixtures of components are introduced in to a HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slower.

The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated.

Most of the drugs in multicomponent dosage forms can be analyzed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC method eliminates tedious extraction and isolation procedures.

Mechanism of separation[14]

Reversed phase chromatography is performed on a nonpolar stationary phase with a polar mobile phase. In RPLC, solute retention is mainly due to hydrophobic interactions between the solutes and the nonpolar hydrocarbon stationary surface. The nonpolar components of a sample interact more with the relatively nonpolar hydrocarbon column packing and thus elute later than polar components. In NPLC, many types of interaction have been described, but hydrogen bonding often predominates.

The elution order of solutes in RPLC is in the order of decreasing polarity, i.e., increasing hydrophobicity, while in NPLC the least polar compound elutes first. Thus, NPLC and RPLC are complementary in elution order. The general theory to describe separation is, however, similar. The analyte is capable of adsorbing to and desorbing from the stationary phase, so it migrates through the column more slowly than the solvent. The width of the analyte band is limited at a theoretical minimum by the volume in which the analyte is injected and is increased by processes that tend to disperse the band, such as diffusion of the analyte in the mobile phase.

In HPLC, the type and composition of the eluent is one of the variables influencing the separation.Despite the large variety of solvents used in HPLC, there are several common properties:

Purity

Detector compatibility

Solubility of the sample

Low viscosity

Chemical inertness

For normal phase mode, solvents are mainly nonpolar; for reversed-phase, eluents are usually mixture of water with some polar organic solvent such as acetonitrile or methanol.

Size-exclusion HPLC has special requirements. SEC eluents have to dissolve polymers, but the most important is that SEC eluent has to suppress possible interactions of the sample molecule with the surface of the packing material.

Instrumentation[3,13,15]

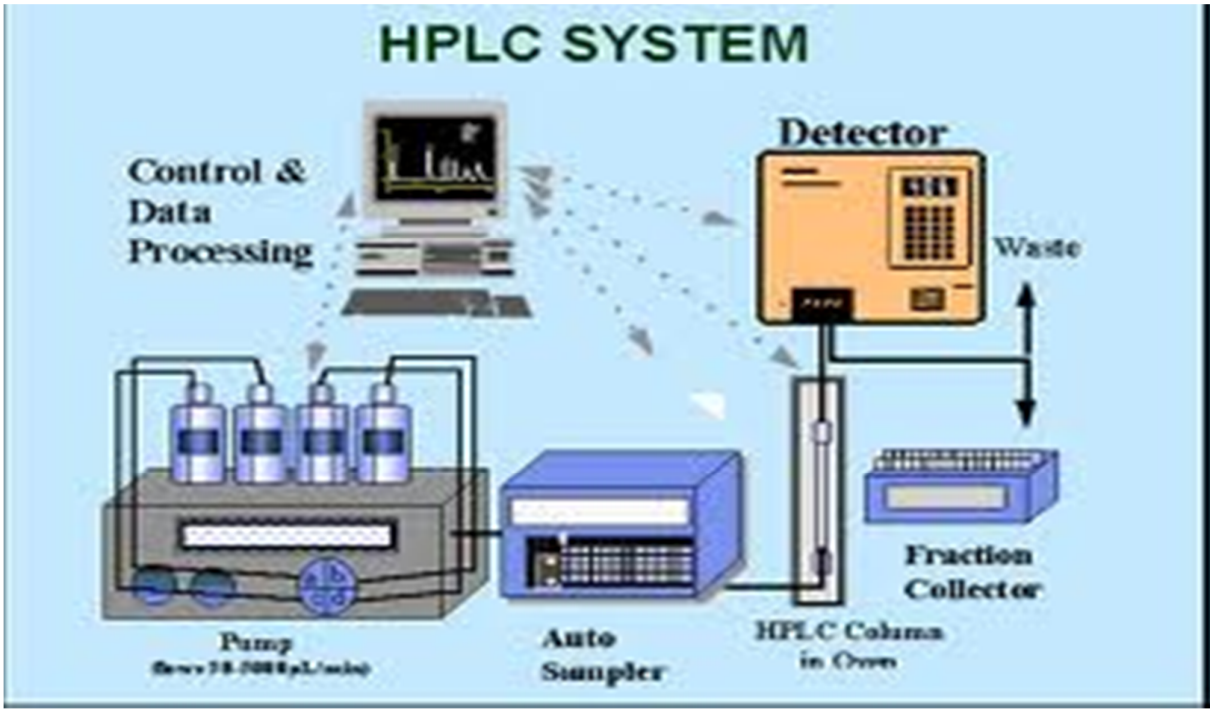


Fig-14[44]

Modern hplc instruments requires several hundreds of atmospheres to achieve reasonable flow rates. Because of these high pressures the equipment in hplc is more elaborate n expensive.It consists of

1Mobile-phasereservoirs and solvent systems A modern hplc apparatus is equipped with one or more glass reservoirs. Provisions are often included to remove dissolved gases and dust. Degassers may consists of a vaccum pumping system, a distillation system,a device heating and stirring. A system for sparging is also incorporated, in which the dissolved gases are swept out of solution by fine bubbles of inert gas. An elution may be of two types isocratic in which a solvent or mixture of constant composition is used, And gradient which consists of two solvents that differ significantly in their polarities and used in varied composition during the separation.

2 Pumping system The pumping system is one of the most important features of an HPLC system. There is high resistance to solvent flow due to the narrow columns packed with small particles, and high pressures are therefore required to achieve satisfactory flow rates.

The main feature of a good pumping system is that it is capable of output of at least 3.4×10^7 Pa (5000 p.s.i) and ideally there must be no pulses of flow through the system. There must be a flow delivery of at least 10 cm^3 min^{-1} for normal analysis and upto 30 cm^3 min^{-1} for preparative analysis. All material in the pump should be chemically resistance to all solvents. Various pumping systems are available

Pneumatic Pumps

They are mostly used for preparative purpose or chosen for the slurry packing of column. It uses the pressurized gas as source to drive the mobile phase to the column.

Syringe Type Pumps

It works on the principle of positive solvent displacement by a piston mechanically driven at a constant rate in a piston chamber of about 250-500 ml capacity with the generation of pulseless flow with high pressure capabilities (200-475 atm).

Reciprocating Pumps

This is generally electrically driven; the driven piston pressurizes the eluent. In this type the movement of plunger piston causes the suction of the eluent during the backward stroke and the delivery of the pressurized solvent during the forward stroke.

Hydrualic pumps

These pumps also work on the same principle as that of the pneumatic pumps, but use liquid pressures rather than gas pressures.

During the chromatographic experiment, a pump can deliver a constant mobile phase composition (isocratic mode) or a variable mobile phase composition (gradient mode).

which operate on the principle of constant pressure or constant displacement.

Constant pressure pumps

It produce a pulseless flow through the column, these pumps operate by the introduction of high pressure gas into the pump, and the gas in turn forces the solvent from the pump into the column.

Constant displacement pumps

It maintains a constant flow rate through the column irrespective of changing conditions within the column. One form of constant displacement pump is a motor-driven syringe type pump where a fixed volume of solvent is forced from the pump to the column by a piston driven by a motor.

Modern pumps have the following parameters:

Flow rate range: 0.01 to 5 mL/min

Flow rate stability: not more than 1%

For SEC flow rate stability should be less than 0.2%

Maximum pressure: up to 300 hPa

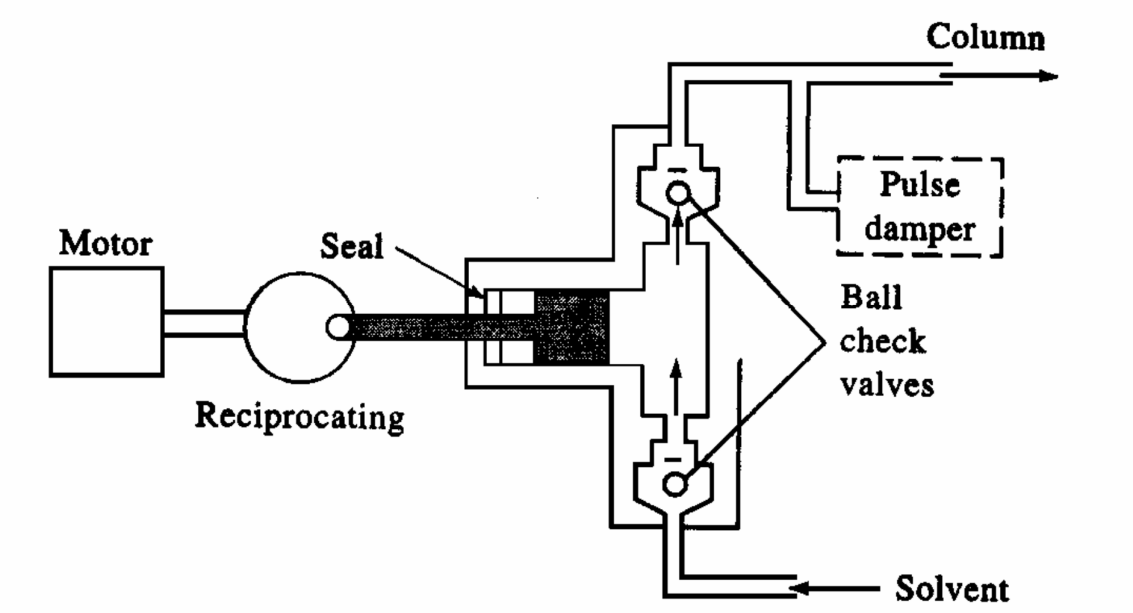


Fig-2[42]

Pumping system

3 Sample injection systems The sample introduction system should enable the sample to be introduced as a narrow plug onto the column so that peak broadening is negligible. Two means for analyte introduction on the column are injection into a flowing stream and a stop flow injection. These techniques used can be fixed volume valve injection, variable valve injection or septum injection. The injector serves to introduce the liquid sample into the flow stream of the mobile phase. Typical sample volumes are 5-20 microliters (µl). The injector must also be able to withstand the high pressures of the liquid system.

Manual Injector manually loads sample into the injector using a syringe and then turns the handle to inject sample into the flowing mobile phase which transports the sample into the beginning (head) of the column, which is at high pressure.**An autosampler** is the automatic version, used when the user has many samples to analyze or when manual injection is not practical. Automatic injector is a microprocessor-controlled version of the manual universal injector. Usually, up to 100 samples can be loaded into the auto injector tray.

The system parameters such as flow rates, gradient run time, volume to be injected etc. are chosen, stored in memory and sequentially executed on consecutive injections.

4 Column

Columns are considered as the “heart of the chromatograph”. The column’s stationary phase separates the sample components of interest using various physical and chemical parameters. The small particles inside the column are what cause the high backpressure at normal flow rates. The pump must push hard to move the mobile phase through the column and this resistance causes a high pressure within the chromatograph.

Types of Columns used in HPLC

Analytical [internal diameter (i.d.) 1.0 - 4.6-mm; lengths 15 –250 mm]

Preparative [i.d.> 4.6 mm; lengths 50 –250 mm]

Capillary [i.d. 0.1 -1.0 mm; various lengths]

Nano [i.d.< 0.1 mm, or sometimes stated as < 100 µm]

Materials of construction used for the tubing

Stainless steel (the most popular; gives high pressure capabilities)

Glass (mostly for biomolecules)

PEEK polymer (biocompatible and chemically inert to most solvents)

Column-Packing Materials

HPLC columns are either polymers or silica based. Silica based packings are the most popular and often used systems. The chemical natures of these packings vary with the stationary phase; their physical characteristics are similar. The average particle diameter of the packings is between 3 to 10 µ with a narrow size distribution. The columns of smaller particles permit faster separation than columns of larger particle size. Higher strength particles provide columns that exhibit lower back pressures and longer lifetime. The silica surface contains various kinds of SiOH (silanol) groups and those particles heated at high temperatures, e.g., 800°C, are devoid of such groups and hence cannot be used for columns.

Free silanols are undesirable for the separation of the basic molecules. The addition of appropriate alkali can eliminate problems of secondary interactions between the basic compounds and acidic silanols (e.g; triethylamine). The secondary reaction between the acidic solutes can also be prevented by the addition of small amount of carboxylic acid into the mobile phase.

Most bonded phase silica packings are made surface-reacted organosilanes. Various alkyl and alkyl substituted silica are made with this reaction. A few packings use a polymerized surface layer that results from the reaction of di or tri functional silanes with silica particles. The stability of the bonded phase is important in HPLC reproducibility. Long chain alkyl bonded phase packings (e.g C_8 or C_{18}) generally are more stable than monomeric phases (e.g, diols). End capping is often used to more completely bond (silanize) packings and consists of a subsequent reaction with trimethylchlorosilane or hexamethyldisilane, to increase the cover support and to minimize unwanted reaction with free silanols.

Reversed phase separations can be made using polymer (polystyrene) particles, which are spherical and porous. Such polymeric (nonsilica) particles can withstand solutions with pH 2 to 13, where silica columns degrade gradually, normally reverse phase separations can be made with C_8, C_{18}, C_3, C_4, phenyl ethyl, cyano, amino, polystyrene packings while normal phase separations must utilize cyano, diol, amino and pure silica packings.

.

Fig-3[41]

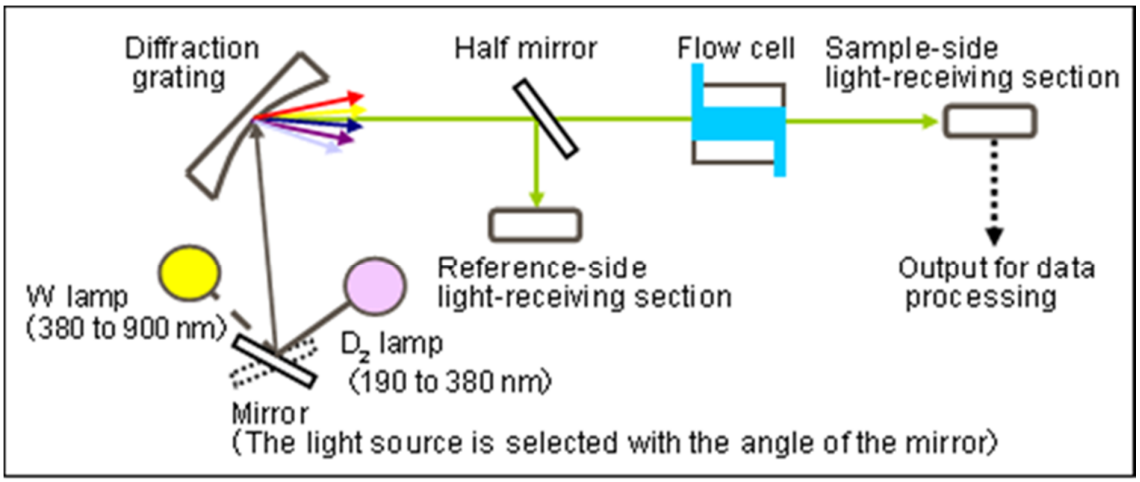
5 Detectors Many different detectors are used in RPLC, including ultraviolet-visible spectrophotometers (UV-VIS), refractive index (RI) detectors, electrochemical (EC) detectors, evaporative light-scattering detectors, fluorimeters, and others. The function of the detector in HPLC is to monitor the mobile phase as it emerges from the column. The detector provides an output to a recorder or computer that result in the liquid chromatogram.

Generally, HPLC detectors are classified as ***Bulk Property Detector*** which detect based on differential measurement of a property, which is common to both the sample and the mobile phase and **Solute Property Detector** which respond to a physical property of the solute, which is not exhibited by the pure mobile phase.

The various detectors are detailed below

UV/UV-VIS Detectors:-

The most widely used detector in HPLC is the UV-absorption spectrophotometer detector. In this detector the changes in UV-absorption when the solution passes through a flow cell is measured.UV detectors are concentration sensitive and have the advantage that they don't destroy the solute. The continuous emission of energy by deuterium lamp as a light source, with the wavelength of its light ranging from 190 to 380 nm can be utilized in conjunction with a monochromator to provide a variable wavelength detector. The variable wavelength facility is extremely useful for getting better sensitivity in difficult analysis as solutes can be monitored at their wavelength of maximum absorption. If components are to be detected at wavelength longer than this, a UV-VIS detector is used, which employs an additional tungsten lamp (W lamp).The stability of current detector is such that the concentrations of few nanograms can be measured. Different components have a different spectrum. Components with a large molar extinction coefficient can show a large peak even in small amounts. Not all molecules possess sufficiently strong UV chromophore for satisfactory UV absorption. Bile acids, lipids, sugars etc., are examples of such compounds. Today such molecules are detected by the process of derivatisation when a chromophoric group is attached to the solute either at pre-column or post column level.



Diagrammatic illustration of a UV-VIS detector optical system

Fig-4[43]

Diode Array Detector (DAD)/ Photodiode Array Detector (PDA)

Photodiode arrays or semiconductor devices are used in the detection unit. A DAD detects the absorption in UV to VIS region. While a UV-VIS detector has only one sample-side light-receiving section, a DAD has multiple photodiode arrays to obtain information over a wide range of wavelengths at one time, which is a merit. If the measurement is performed at a fixed wavelength, components are identified from only their retention time; thus, a minor deviation in retention time can make identification of components difficult. In such a case, the DAD can be used to identify components by a comparison of the spectrum.

Fluorescence Detector

A fluorimetric detector is extremely sensitive when compared to UV-Visible detector and works on the principle that the fluorescent energy emitted at a longer wavelength, known as emission wavelength, is proportional to the concentration. First, however the solute has to be excited with the excitation wavelength energy, this wavelength is always lower than the emission wavelength, but solute has to possess initial fluorescence. Analytes with no fluorescence can react with compounds, which are fluorescent derivatizing agents, as in UV detectors to produce fluorescence.It allows to quantify and identify compounds and impurities in complex matrices at extremely low concentration levels (trace level analysis).

Differential Refractive Index (RI) Detector

The ability of a compound or solvent to deflect light provides a way to detect it. The RI is a measure of molecule's ability to deflect light in a flowing mobile phase in a flow cell relative to a static mobile phase contained in a reference flow cell.The amount of deflection is proportional to concentration.The RI detector is considered to be a universal detector but it is not very sensitive. Any component in the eluate can be detected; thus, the RI detector is often called a "universal detector". This detector is at least three times as sensitive as the UV-visible detector, but variations in ambience produce several fluctuations.

Conductivity Detector

Electrochemical detectors also very sensitive, but its usage is restricted to only oxidation and reductions. The system works on the principal of polarography. A serious drawback of this detector is that the electrodes get contaminated and poisoned by absorption of the oxidized or reduced compounds. A conductivity detector is employed as a detector in an ion chromatograph, which is a system dedicated to measuring ions. This detector is used mainly to measure inorganic ions and small organic substances, including organic acids and amines. The conductivity detector is highly sensitive, but very susceptible to the effect of temperature variation (a change of 1ºC in solution temperature causes a change of roughly 2% in electric conductivity). Various methods of avoiding temperature variations have been devised, such as constant-temperature cells.

Evaporative Light Scattering Detector (ELSD)

An ELSD atomizes the column eluate, shines light on the resulting particulate components, and detects the resulting scattered light. Theoretically, an ELSD can detect any nonvolatile component. An ELSD has sensitivity roughly 10 times higher than an RI detector, but has a slightly low sensitivity to low molecular components due to their small size. An ELSD is used mainly to detect non-UV-absorbing components. Attention should be given to the fact that nonvolatile salts cannot be used as the eluent.

Charged Aerosol Detector

Like an ELSD, CAD atomizes the column eluate to make the sample components particulate. However, a CAD detects them electrically by ionizing them with charged N_2 gas. A merit of the CAD is the ability to detect components with sensitivity higher than that of an ELSD, with a sensitivity which depends only slightly on component. The eluent for a CAD is similar to that for ELSD.In addition, the LC-MS system, in which the components separated by HPLC are further analyzed using a mass spectrometer, is becoming widely used because of its high sensitivity and the possibility of specific detection.

Data Recorder

Frequently called the data system, the computer not only controls all the modules of the HPLC instrument but it takes the signal from the detector and uses it to determine the time of elution (retention time) of the sample components (qualitative analysis) and the amount of sample (quantitative analysis

PREFFERED EXPERIMENTAL CONDITIONS FOR THE INITIAL HPLC SEPERATION[16]

TABLE 2

Separation variable	Preferred Initial choice
Column Dimensions Particle size Stationary phase	 15*0.46 5micro m C8 or C18
Mobile phase Solvents A and B %B Buffer (compound, pH, concentration)	 Buffer- Acetonitrile 80-100% 25mM potassium phosphate 2.0 pH 3.0
Additives (amine modifiers, ion-pair reagents)	Do not use initially
Flow rate	1.5-2.0ml/min
Temperature	35-45c
Sample size Volume Weight	 Less than 25micro Less than 100 micro g

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY[17]

Thin layer chromatography (TLC); also known as planar-chromatography or flatbed chromatography is like all other chromatographic techniques, a multi-stage distribution process. HPTLC is the most simple separation technique today available to the analyst. It can be considered a time machine that can speed your work and allows you to do many things at a time usually not possible with other analytical techniques.

Chromatography is a powerful separation technique that finds application to all branches of science [18]. Chromatography is a separation technique whereby the components of a mixture may be separated by allowing the sample to be transported through packed bed of material by fluid mobile phase [19,20]. Out of almost 700 pharmaceutical formulations that are documented in the USP almost 43% of theprocedures are those documented by thin layer chromatography.

Features of HPTLC

1. Simultaneous processing of sample and standard - better analytical precision and

accuracyless need for Internal Standard

2. Several analysts work simultaneously

3. Lower analysis time and less cost per analysis

4. Low maintenance cost

5. Simple sample preparation - handle samples of divergent nature

6. No prior treatment for solvents like filtration and degassing

7. Low mobile phase consumption per sample

8. No interference from previous analysis - fresh stationary and mobile phases foreach analysis - no contamination

Table-3

Main difference of HPTLC and TLC [14]

PARAMETER	HPTLC	TLC
Layer of Sorbent	100µm	250µm
Efficiency	High due to smaller particle size generated	Less
Separations	3 - 5 cm	10 - 15 cm
Analysis Time	Shorter migration distance and the analysis time is greatly reduced	Slower
Solid support	Wide choice of stationary phases like silica gel for normal phase and C8 , C18 for reverse phase modes	Silica gel, Alumina & Kiesulguhr
Development chamber	New type that require less amount of mobile phase	More amount
Sample spotting	Auto sampler	Manual spotting
Scanning	Use of UV/ Visible/ Fluorescence scanner scans the entire chromatogram qualitatively and quantitatively. The scanner is an advanced type of densitometer.	Not possible

INSTRUMENTATION[46]

1.Platecoaters

2.Drying racks

3.Platecutters

4.Immersion device

5.Plateheater

6Sample application

7.Development chamber

8.Derivatization devices

9.Scanning densitometer

1.Platecoate.Handoperated•The manual plate coater functions in the same manneras the automatic coater , exceptwith this model the plates are pushed through by hand , one after the other and lifted off on the other slide.

1Automatic Plate Coater The glass plates to be coated are conveyed underneath a hopper filled with the adsorbent suspension.The plates are moved by a motorizedconveying system at a uniform feedingrate of 10cm/s, to ensure a uniform speed.

2.Drying Rack The Drying Rack consists of ten individual aluminum trays. A tin box for storing the trays and twowire handles , to move the stack while hot ,are supplied. The drying rack is convenient to use ,particularly when TLC plates are preparedwith the automatic plate coater in large runs.

3.Plate Cutter Used to cut HPTLC plates easily and more precisely. Cuts plates with a thickness up to 3mm.Does not damage the sensitive layer. Easy to handle .Read the required size from the scale directly. Helps saving costs on pre-coated platesof high quality by preventing off cuts.

4.ImmersionDevice For proper execution of the dipping technique , the chromatogram must be immersed and withdrawnat a controlled uniform speed.Uniform vertical speed Immersion time selectable between 1 and 8 seconds.The device can be set to accommodate 10cm and 20cm plate height. Battery operated , independent of power supply.

5.PlateHeater The TLC plate heater is designed for heating TLC platesto a given temperature , while ensuring homogenous heating across the plate. The TLC plate heater has a heating surfacewhich is resistant to all common reagents and is easily cleaned. Programmed and actual temperature are digitallydisplayed.The temperature is selectable between 25 and 2000c.The plate heater is protected from overheating. 13

6.SampleApplication Usual concentration of applied samples 0.1 to 1 µg / µl for qualitative Analysis and quantity may vary in quantitation based on UV absorption 1 to 5 µl for spot and 10 µL for band application.

Manual, Semi-Automatic, Automatic Application

Manual with calibrated capillaries.

Semi and auto-application through applicators.

Applicators use spray on or touch and deliver technique for application.

7. Developing Chamber

1.Twin trough chamber

2. Automatic developing chamber

1.Twin Trough Chamber

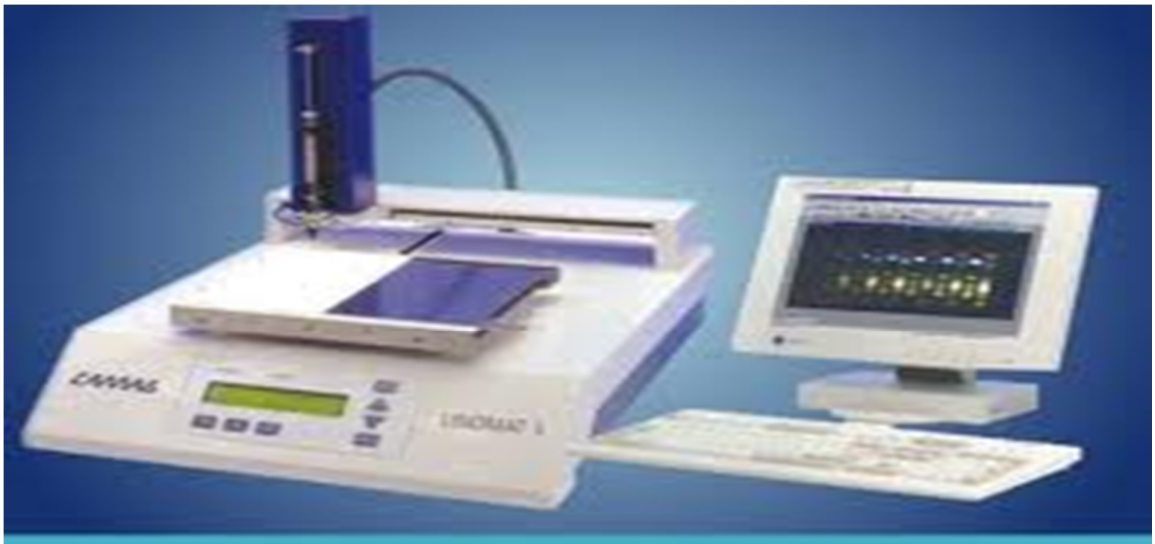
Low solvent consumption:20 mL of solvent is sufficient for the development of a 20x20cm plate. This not only saves solvent , but also reduces the waste disposal problem

Reproducible pre –equilibrium with Solvent vapor: For pre-equilibration, the TLC plate is placed in the empty troughopposite the trough which contains the pre-conditioning solvent. Equilibration can be performed with any liquid and for any period of time.

Start of development : It is started only when developing solvent is introduced into the trough with the plate.

2.Automaticdeveloping chamber(ADC) In the ADC this step is fully automatic and independent ofenvironmental effects.The activity and pre-conditioning of the layer, chamber saturation,developing distance and final drying can be pre-setand automatically monitored by ADC.

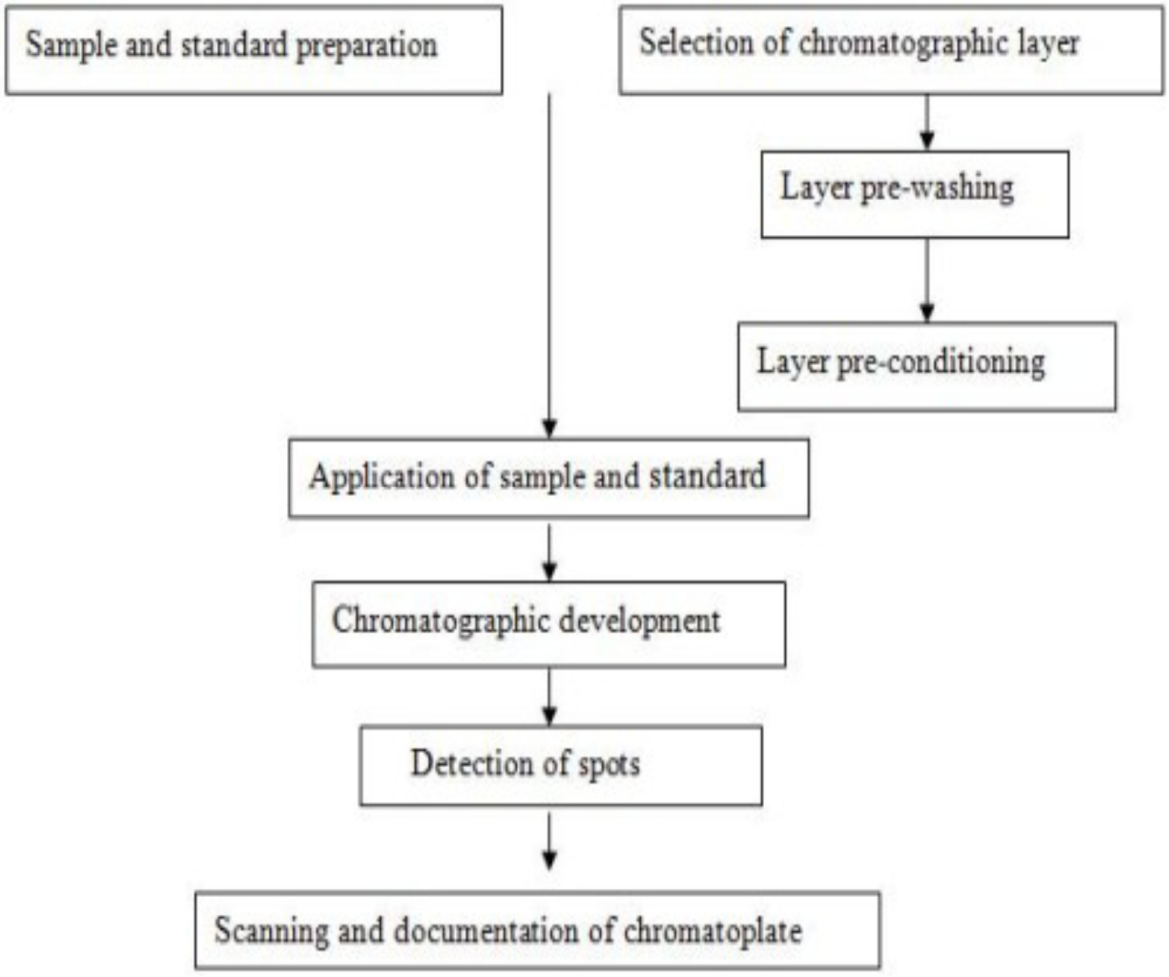
Fig5[45]



The High Performance Thin Layer Chromatography (HPTLC) equipment

STEPS INVOLVED IN HPTLC METHOD DEVELOPMENT

FIG 6



Selection Of ChromatographicLayer[22]

Precoated plates - different support materials - different Sorbents available

80% of analysis - silica gel GF · Basic substances, alkaloids and steroids – AluminumOxide,Amino acids, dipeptides, sugars and alkaloids - cellulose

Non-polar substances, fatty acids, carotenoids, cholesterol - RP2, RP8 and RP18

Preservatives, barbiturates, analgesic and phenothiazines- Hybrid plates-RPWF254

Selection Of HPTLC Plates

Previously hand made plates is used in TLC for both qualitative andquantitative work. Certain drawbacks with that is non-uniform layer, formation of thick layer paved for advent of precoated plates.

Nowadays precoated plates are available in different format and thickness by various manufactures. Pre coated plates can be used for both qualitative and quantitative work in HPTLC.

Glass plates

Poly ester/polyethylene

Aluminum plates

Glass Plates

Resistant to heat & Chemical

Easy to handle

Fig7

Fragile

High weight

Higher production cost

Fragile

High weight

Higher production

Poly ester /Poly ethylene

Thickness of Plate 0.2 mm Figure-9

It can be produced in Roll forms

Unbreakable

Less packing material required

Development of plate can not be above temp. 1200 losses of it shape

Aluminium plates

Thickness of Plate 0.1 mm

It can be produced in Roll forms

Sorbents Used In HPTLC Plates

Sorbent, which are used in conventional TLC, are also used in HPTLC with or without modification

Silica gel 65F(modified)

Highly purified Silica gel 60

Aluminium oxide

Cellulose Microcrystalline

Slica gel

Reversed stationary phase

Hybrid Plates

Particle SizeOf Sorbents

Layer Thickness

HPTLC 6μm, TLC 10μm.

The layer of thickness in HPTLC is around 100-200 μm,where as 250μm in conventional TLC.

Layer Pre -Washing

Ascending method

Dipping method

Continuous method

Solvents used for pre washing

Methanol (commonly used)

Chloroform: Methanol: Ammonia (90:10:1)

Chloroform: Methanol (1:1)

Methylene chloride: Methanol (1:1)

Ammonia solution (1%)

Sample and Standard Preparation

To avoid interference from impurities and water vapours

Low signal to noise ratio - Straight base line- Improvement of LOD

Solvents used are Methanol, Chloroform: Methanol (1:1), Ethyl acetate: Methanol (1:1), Chloroform: Methanol: Ammonia (90:!0:1), Methylene chloride : Methanol (1:1), 1% Ammonia or 1% Acetic acid

Dry the plates and store in dust free atmosphere

Sample Preparation

Proper sample preparation is an importantprereqisite for success of TLC separation. Besides maximizing the yield of analyte in selected solvent, stability of analyte during extraction and analysis must be considered. Therefore choice of suitable solvent for given analysis is very important.

Solvent for dissolving sample should be non polar and volatile as far as possible, since polar solvents are likely to induces circular chromatogram at the origin.

Activation Of Pre coated Plates

The plates are activated by placing in an oven at 110-120°c for 30 min., this step will removes water that has been physically absorbed on surface at solvent layer. Freshly opened box of HPTLC plates usually does not require activation. Activation at highertemp.and for longer time is avoided which pleads to very active layer and there is risk of sample being decomposed.

Application Of Sample And Standard Solution

Sample application is one imp. And critical step for obtaining good resolution for quantification by HPTLC. Sample / std. Is applied as a sporty or band depending upon the analysis.

Spot application is done by using

Capillary tubes

Micro bulb pipettes

Micro syringes

Automatic sample applicator

Selection of mobile phase[21]

Trial and error

one's own experience and Literature

Normal phase

Stationary phase is polar. Mobile phase is nonpolar. Non-polar compounds eluted first because of lower affinity with stationaryphase.Polar compounds retained because of higher affinity with the stationary phase.

Reversed phase

Stationary phase is no polar**.** Mobile phase is polar

Polar compounds eluted first because of lower affinity with stationary phase.Non-Polar compounds retained because of higher affinity with the stationary phase.3 - 4 component mobile phase should be avoided.Multi component mobile phase once used not recommended for further use andsolvent composition is expressed by volumes (v/v) and sum of volumes isusually 100. Twin trough chambers are used only 10 -15 ml of mobile phase is required.

Components of mobile phase should be mixed introduced into the twin – troughchamber.

Pre- conditioning (Chamber saturation)

Un- saturated chamber causes high Rf values.

Saturated chamber by lining with filter paper for 30 minutes prior to development -uniform distribution of solvent vapours - less solvent for the sample to travel - lower Rfvalues.

Chromatographic development and drying

After development, remove the plate and mobile phase is removed from the plate – toavoid contamination of lab atmosphere

Dry in vacuum desiccator - avoid hair drier - essential oil components may evaporate

Chromatogram Development

After application of sample in HPTLC plate, chromatogram is developed by dipping in suitable solvent system taken in a developing chamber. The solvent system is rises over the layer by capillary action and separation of sample in to different components takes Place.

Selection of solvent system / mobile phase

Chamber saturation

Type of development and developing device.

Linear & Radial Development

In a close bed tec. Such as HPLC only Linear development

Is possible, But an open bed tec. Like HPTLC does not suffer this limitation.

HPTLC can develop by

Ascending (linear),Circular,Anti- circular

Detection and visualization

Detection under UV light is first choice - non destructive

Spots of fluorescent compounds can be seen at 254 nm (short wave length) or at 366nm (long wave length)

Spots of nonfluorescent compounds can be seen - fluorescent stationary phase is used -silica gel GF

Non UV absorbing compounds like ethambutol, dicylomine etc. - dipping the plates in0.1% iodine solution

When individual component does not respond to UV - derivatisation required fordetection

There is no difficulty in detecting the colored substances or colorless substancesabsorbing UV-radiations or with fluoresce (Riboflavin)

“Derivatisation”

Detection of spots / bands are done by

Destruction / Non-reverse

Non-destructive / Reversible

Miscellaneous methods

Quantification

Sample and standard should be chromatographed on same plate - after development chromatogram is scanned

Camag TLC scanner III scan the chromatogram in reflectance or in transmittance mode by absorbance or by fluorescent mode - scanning speed is selectable up to 100 mm/s - spectra recording is fast - 36 tracks with up to 100 peak windows can be evaluated.

Calibration of single and multiple levels with linear or non-linear regressions are possible · When target values are to be verified such as stability testing and dissolution profile single level calibration is suitable Statistics such as RSD or CI report automatically

Concentration of analyte in the sample is calculated by considering the sample initially taken and dilution factors.

VALIDATION

Validation of an analytical method is the process by which it is estimated, bylaboratory studies, that the performance characteristics of the method meet therequirements for the intended analytical applications [28]

REASONS FOR VALIDATION[14.15]

1. Enables scientists to communicate scientifically and effectively on technical matters.
2. Setting standards of evaluation procedures for checking complaints and taking remedial measures.
3. Retrospective validation is useful for trend comparison of results compliance to cGMP/cGLP.
4. Closer interaction with pharmacopoeia harmonization particularly in respect of impurities determination and their limits.
5. For taking appropriate action in case of non – compliance.
6. To provide high degree of confidence that the same level of quality is consistently built into each unit of finished product from batch to batch.
7. Economic: The consistency and reliability of validated analytical procedure is to produce a quality product with all the quality attributes, thus providing indirect cost saving from reduced testing or re testing and elimination of product rejection. As quality control process is not static,

SUMMARY OF VALIDATION PROCEDURE

PARAMETERS USED FOR ASSAY VALIDATION:-[7,24,25]

Specificity[23]

Specificity is the ability to assess unequivocally the analyte in the presence ofcomponents which may be expected to be present. Lack of specificity of an individualanalytical procedure may be compensated by other supporting analytical procedures.

Accuracy[2]

The ICH defines the accuracy of an analytical procedure as the closeness of agreement between the values that are accepted either as conventional true values or an accepted reference value and the value found.

Accuracy is usually reported as percent recovery by assay, using the proposed analytical procedure, of known amount of analyte added to the sample. The ICH also recommended assessing a minimum of nine determinations over a minimum of three concentration levels covering the specified range (e.g., three concentrations/three replicates).

Precision

The precision of an analytical procedure expresses the closeness of agreementbetween a series of measurements obtained from multiple sampling of the samehomogenous sample under the prescribed conditions. Precision of an analyticalprocedure is usually expressed as the variance, standard deviation or coefficient of some form of validation .

Precision is usually investigated at three levels: **repeatability, intermediate precision, and reproducibility.**

Linearity[27]

Linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Range

Range of an analytical procedure is the interval between the upper and lower concentration (amount) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Robustness[25]

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in the analytical procedure parameters. The robustness of the analytical procedure provides an indication of its reliability during normal use. The evaluation of robustness should be considered during development of the analytical procedure. Common variations that are investigated for robustness include filter effect, stability of analytical solutions, extraction time during sample preparation, pH variations in the mobile-phase composition, variations in mobile-phase composition, columns, temperature effect, and flow rate.

Ruggedness[16]

It is defined as the reproducibility of results when the method is performed under actual use conditions. This includes different analysts, laboratories, columns, instruments, sources of reagents, chemicals, solvents, and so on. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method.

Detection limit

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Based on the standard deviation of the response and the slope, the detection limit (DL) may be expressed

$$DL = \frac{3\sigma}{S}$$

Where

σ = standard deviation of the response,

S = slope of the calibration curve (of the analyte).

Quantitation limit

The quantitation limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision, accuracy,and reliability by the proposed method.

Based on the standard deviation of the response and the slope, quantitation limit may be expressed as

$$QL = \frac{10\sigma}{S}$$

Where

σ = standard deviation of the response,

S = slope of the calibration curve (of the analyte).

System suitability testing [29]

System suitability is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such.

The system suitability testing parameters established for the liquid chromatographic procedure are:

Retention time (Rt)

This is the time of emergence of the maximum of a component after injection.

Symmetry factor (or) tailing factor (T)

$$T = \frac{W0.05}{2f}$$

The assessment of peak shape is in terms of symmetry factor.

Number of theoretical plates (N)

$$N = 5.54\left(\frac{t_R}{W_{h/2}}\right)^2$$

The assessment of performance of efficient of a column is in terms of the number of theoretical plates.

Resolution

Resolution is a measure of the extent of separation of two components and the baseline separation achieved. Resolution is generally defined as "the distance between the centers of two eluting peaks as measured by retention time or volume divided by the average width of the respective peaks".

$$R = \frac{2(t2-t1)}{W2+W1}$$

Statistical parameters

Statistics consist of a set of methods and rules for organizing and interpreting observations.

The precision or reproducibility of the analytical method was determined by repeating the analysis six times and the following statistical parameters were calculated.

The Formulas are

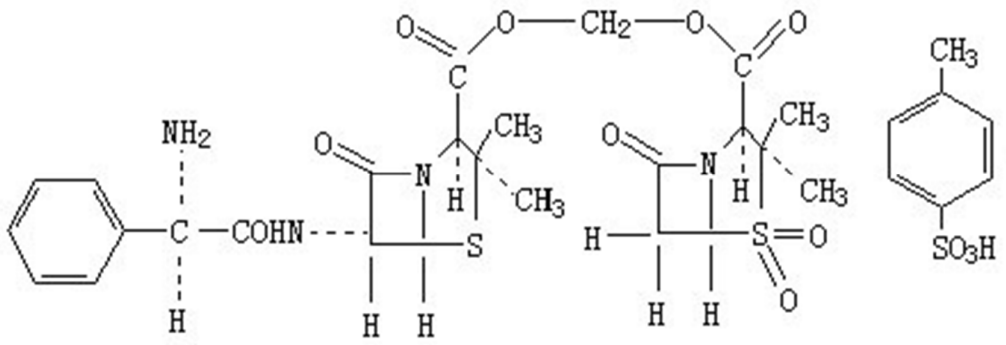
$$\text{Standard Deviation} = \frac{\Sigma(x - x)^2}{n - 1}$$

$$\text{R.S.D (\%)} = \frac{\text{S.D X 100}}{\text{Mean}}$$

Drug profile

Drug profile

Structure



Chemical name	(2S,5R)-(3,3-dimethyl-4,4,7-trioxo-4-thia-1-azabicyclo[3.2.0]hept 2ylcarbonyloxy) methyl (2S,5R,6R)-6-[(R)-2-Amino-2-phenylacetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylate;Hydroxymethyl(+)-(2S,5R,6R)-6-[(R)-(2-amino-2phenylacetamido)]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylate,(2S,5R)-3,3-dimethyl-7oxo-4-thia-1-azabicyclo[3.2.0]heptane-2 carboxylate 4,4 dioxide; 4 methylbenzenesulphonic acid
Molecular formula	$C_{32}H_{38}N_4O_{12}S_3$
Molecular weight	766.85 g/mol
Physical appearance	White to off whitecrystallinepowder

Solubility	Practically insoluble in water, sparingly soluble in alcohol and soluble in methanol
Category	β-lactam Antibiotic
Mode of action	Sultamicillin has a wide spectrum of activity

Spectrum of activity[33,35,37,30]

Gram-positive bacteria	**Gram-negative bacteria**	**Anaerobic bacteria**
Staphylococcus aureus (β-lactamase-producing and non-producing)	Haemophilusinfluenzae (β-lactamase-producing and non-producing)	Bacteroides species (including Bacteriodesfragilis)
Staphylococcus epidermidis	Moraxella catarrhalis	Peptococcus species
Streptococcus faecalis	Escherichia coli	Peptostreptococcus species
Streptococcus pneumonia	Klebsiella	Clostridium species
Streptococcus pyogenes	Proteus mirabilis	
Streptococcus viridians	Neisseria gonorrhoeae	
	Proteus vulgaris	
	Providencia rettgeri	
	Providencia stuartii	
	Morganellamorganii	

Table 4

It is a prodrug of ampicillin and of the beta-lactamase inhibitor sulbactam; it consists of two compounds linked as a double ester.[37]During absorption from the gastrointestinal tract it is hydrolysed, releasing equimolar quantities of ampicillin and sulbactam. Provides high serum concentrations following IM and IV administration.Ampicillin is 28% reversibly bound to serum proteins and sulbactam is 38% reversibly bound

Mechanism of action [31]

Sultamicillin is a combination of ampicillin and sulbactam. It exerts bactericidal effect by inhibiting bacterial wall mucopeptide biosynthesis mediated by the ampicillin and also by the irreversible β–lactamase inhibition by sulbactam in its formula. The addition of sulbactam to ampicillin extends the spectrum of ampicillin to include some β-lactamase producing organisms or some penicillin resistant species thereby inhibiting bacterial cell wall synthesis by binding to one or more of the penicillin binding proteins (PBPs) which in turn inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls, thus inhibiting cell wall biosynthesis. Bacteria eventually lyse due to ongoing activity of cell wall autolytic enzymes like autolysins and murein hydrolases while cell wall assembly is arrested.

Uses

Skin and soft tissue infections.

Intra-abdominal infections.

Gynecological infections.

Respiratory and urinary tract infections.

Gonococcal infections.

Bacterial septicemia.

Bone and joint infections.

Prophylaxis following abortion and cesarean section to decrease the risk of sepsis.

Contraindications and Cautions

Sultamicillin is contraindicated in individuals hypersensitive to penicillin's and cephalosporins.Ampicillin should not be used in patients with infectious mononucleosis since the drug causes serious skin rashes in these cases.

During prolonged treatment periods, vital organs functions including renal, hepatic and hematopoietic system should be monitored. Though ampicillin and sulbactam have no carcinogenic or mutagenic effects, its safety of administration in pregnant women has to be established.

Development of super infection during administration should be considered and if it occurs treatment should be discontinued and necessary measures should be applied.

Special precautions should be taken for neonates, pregnant women and lactating mothers.Caution should be taken for patients with impaired renal function and in patients suffering from hyperuricemia.

Adverse effects

Local (at the injection) Pain at the injection site and thrombophlebitis during IV administration.

Gastrointestinal systemsevere nausea or vomiting, stomatitis, tongue discoloration, gastritis, diarrhea, enterocolitis and pseudomembranous colitis.

Skin and soft tissue Skin rash, itching, utricaria, erythema multiformae and rarely exfoliative dermatitis and some hypersensitive reactions.

HematologicalAgranulocytosis and decrease in hemoglobin, hematocrit, erythrocyte, leucocyte, lymphocyte and platelet or increase in lymphocyte, monocyte, eosinophilia and platelet; these findings are reversible and return to normal upon discontinuation of therapy.

Literature review

Literature review on the analytical methods of Sultamicillin tosilate

1. **Friedel, Heather A** *et al.*,**(1989)**presented a review on the anti-bacterial activity**.**Sultamicillin is the tosylate salt of the double ester of sulbactam plus ampicillin. Sulbactam is a semisynthetic [beta]-lactamase inhibitor. Previously the combination hs been using only in the parentral form now a days that has been extended to oral dosage also with the saeneffectas that of parentral one.Sultamicillin has been shown to be clinically effective in non-comparative trials in patients with infections of the respiratory tract, ears, nose and throat, urinary tract, skin and soft tissues, as well as in obstetric and gynaecological infections, and in the treatment of gonorrhoea. In a small number of controlled trials, sultamicillin has shown comparable clinical efficacy to phenoxymethyl penicillin (penicillin V) and to amoxycillin (alone and in combination with clavulanic acid) in the treatment of paediatric streptococcal pharyngitis and acute otitis media, respectively; to cefaclor in the treatment of acute otitis media in adults; and to bacampicillin, cloxacillin and flucloxacillin plus ampicillin in skin and soft tissue infections in adults, children and adult diabetic patients, respectively. Sultamicillin was superior in efficacy to bacampicillin in the treatment of chronic respiratory infections, to cefaclor in the treatment of acute otitis media in adults, and to cefadroxil in the treatment of patients with complicated urinary tract infections. However, in single-dose treatment of uncomplicated gonorrhoea, sultamicillin (1500mg plus probenecid 1g) was inferior to a 2g intramuscular dose of spectinomycin.

2. **Airede.A.K***et al.,*[32]**(1996)** presented a review on theuse of an effective antimicrobial remains a problem in the neonate, thereby necessitating empiric combinations of parenteral agents. We therefore studied oral Sultamicillin's (Unasyn CP-45 899) efficacy and tolerability (dose = 50 mg/kg per day) in the treatment of serious infections in 27 neonates over an 18 month period. The study cohort comprised newborns with suspected or confirmed infections in the Special Care Baby Unit of a referral hospital. The infants with overwhelming/severe infections or proven/suspected renal, hepatic or hematologic disease; or known hypersensitivity to penicillins or any β-lactams were excluded. There were 12 babies with skin and soft-tissue infections, although pneumonia was most predominant in our series. Bacterial isolates were mainly *Staphylococcus aureus, Escherichia Coli* and *Klebsiellapneumoniae* with a β-lactamase production rate of 88%. The clinical cure and improvement rates were 96.3 and 100%, respectively and the

evaluable bacteriologic cure-rate was 93.8%. The mean (S.D.) duration of therapy was 7.4 (2.6) days (range, 4–14) with significant resolution of features occurring within a 48 h period ($^{24}/_{27}$, $P< 0.01$). No serious adverse/side effects were seen as only one (3.7%) experienced mild loose stools. We show with these prospective observations (our cohort albeit small) that sultamicillin orally is efficacious, tolerable and safe for treating of mild to moderate infections in the newborn caused by both gram-positive and gram-negative pathogens.

3. **Changqin H** *et al*.,[33] **(1997)** developed two reversed phase ion pair high performance liquid chromatography for the analysis of Sultamicillin with an ODS column or a CN column. The mobile phase was a combination of methanol and 0.018mol/l tetrabutylammonium hydroxide containing 0.4% of triethylamine whose pH was adjusted to 4.0 by phosphoric acid in the ratio (28:72) for ODS chromatographic system; and acetonitrile - 0.05mol/l tetrabutylammonium hydroxide aqueous solution pH adjusted to 3.5 by phosphoric acid (1:99) in the CN column system. Both chromatographic systems have different characteristics. The ODS system is suitable for determination of the content of Sultamicillin in stability study and the CN column system is more suitable for tracing the changes of impurities in chemical study. Using both the chromatographic systems the stability of Sultamicillin in solution was studied and its degradation profile was discussed.

4 El-Shanawani A.A[34] **.,(1998)**developed a rapid, simple and sensitive method for HPLC determination of Sulbactam, SultamicillinTosylate, Cefaclor, Ampicillin and Cefoperazone in pharmaceutical preparations. The manufacture precursors like 6-aminopenicillanic acid (6APA) in ampicillin or 7-aminocephalosporanic acid (7ACA) in cefaclor and cefoperazone and the expected degradation products like phenylglycin in cefaclor and ampicillin or p-hydroxyphenylglycin in cefoperazone do not interfere with the determination. The drugs were chromatographed on a Spherisorb ODS-2 column with 25% methanol in 0.005M tetramethylammonium hydroxide (TMAH) whose pH was adjusted to 3.4 with 1 M phosphoric acid as mobile phase and salicylamide was used as internal standard. The flow rate was 1 ml/min and detection at 230 nm. This method was applied to Unasyn vials and tablets, Cefobid vials and Ceclor capsules and packets. The relative standard deviation ranged from 1.23 to 2.22%.

5 Pneumologie I *et al*.,[35]**(2001)**reported the emergence of beta-lactamase-mediated resistance to beta-lactam antibiotics among key respiratory tract pathogens has threatened the usefulness of the beta-lactam agents familiar to physicians as being clinically effective and well tolerated. This article reassesses the clinical usefulness of ampicillin when administered in combination with the beta-lactamase inhibitor sulbactam, either intravenously or orally (as the mutual prodrugsultamicillin), in the treatment of upper and lower respiratory tract infections. Numerous clinical studies and several meta-analyses indicate that ampicillin/sulbactam and sultamicillin are clinically effective and well tolerated in both adults and children.

6. Pajchel G *et al.,*[36]**(2002)** explained a micellarelectrokinetic capillaryelectrophoretic method for determination of Sultamicillin in Unasyn oral preparations like tablets and suspensions. Mobile phase used was phosphate-borate buffer containing 1.0% sodium dodecylsulfate with pH 7. The elaborated method ensures separation of Sultamicillin from p-toulenesulphonic acid and impurities like ampicillin, sulbactam and penicillamine. The method was thoroughly validated and the linearity ranged from 0.05- 1.5 mg/ml.

7.ZHang Qing Yuan.,[37]**(2004**) reported Sultamicillin is the tosilate salt of the double ester of sulbactum plus ampicillin. That is hydrolysed by enzymes in the intestinal wall after orally administered, releasing sulbactum and ampicillin in equimolar proportions. Sulbactum is an effective irreversible semisynthetic inhibitor unique for β- lactamase, when in combination with ampicillin, which extends the antibacterial activity of the latter including some β - lactamase - producing strains of bacteria that would otherwise be resistant. The antibacterial activity of sultamicillin against most of bacteria was 2 - 64 times stronger than that of ampicillin. A close relationship exists between the pharmacokinetic handling of both sulbactum and ampicillin. Both time to peak plasma concentration (approximate 1 hour) and profile of elimination are similar for two drugs. Sultamicillin had much higher bactericidal activity than that of ampicillin arid sulbactum(1 : 1). Sultamcillin has been shown to be clinically effective in non - comparative trials in patients with infection of the respiratory tract, nose and throat, urinary tract, shin and soft tissues, as in obstetric and gynecological infections and in the treatment of gonorrhea. The overall incidence of adverse reaction reported was 7.2% (3 005 cases), the typical symptoms with an incidence of greater than 1% was diarrhoea (3. 7%) and soft stools (1. 1%).

8. Bhosle*et al.*,[38] *(2006)* presented a review article where they discussed the mutual prodrug concept and laid a great emphasis on research to discover methods aimed at improving the therapeutic efficacy of drug by minimizing or eliminating their undesirable properties. Mutual prodrug is a type of carrier-linked prodrug where the carrier is another biologically active drug instead of an inert molecule. Sultamicillin is a combination of Ampicillin and Sulbactam with synergistic action. In the design of Sultamicillin, the irreversible β-lactamase inhibitor Sulbactam with very limited antibacterial activity when combined chemically via ester linkage with Ampicillin enhanced its activity both invivo and invitro. Upon oral administration Sultamicillin is completely hydrolysed to equimolar proportions of ampicillin and sulbactam thus forming an efficient prodrug.Sultamicillin results in more efficient oral absorption than its single agent does. Peak serum concentrations of ampicillin achieved are approximately 3.5 fold those obtained with equivalent amount of oral ampicillin. The pharmacokinetic parameters of the two components are similar, both being eliminated primarily by renal excretion. Although the elimination half-lives of both are approximately 1 hr, the high serum concentration achieved coupled with their synergistic activity permit twice daily dosing. One more advantage presented by sultamicillin is that even though most β-lactamase resistant antimicrobials must be given parenterally, sultamicillin is given by mouth. It has been found to be effective against skeletal infections in children, urinary infections in geriatric patients and uncomplicated gonorrhea.

9. H. J. Rogers *et al*[39]., (2009) carried out pharmacokinetic and bioavailability studies of Sultamicillin and estimated it by HPLC. An HPLC assay for sulbactam in plasma, saliva and urine was used to determine the absolute bioavailability of sulbactam and ampicillin from sultamicillin in six normal male volunteers who each received a single oral dose of 750 mg of sultamicillin or iv dose of equivalent amount of ampicillin (441 mg) and sulbactam (294 mg). Treatment was given in random order with not less than four days intervening. The mean peak concentrations and time to peak of sulbactam and ampicillin following the 750 mg oral dose of sultamicillin were 8-9 and 91 mg/l and 0-96 and 0-92 h respectively. The half-lives, systemic and renal clearances for sulbactam and ampicillin were similar. The bioavailability for both drugs from sultamicillin as estimated from both plasma and urine pharmacokinetics was better than 80%. Thus they concluded that sultamicillin is a very efficient prodrug for ampicillin and sulbactam and that the HPLC method was accurate, rapid and easier to perform.

10 .**Mustafa Pola*et al.*,[40](2009**) conducted an electron spin study to study parameters like radiation sensitivity and dosimetric features of Sultamicillin tosylate. Classic sterilization techniques when used leads to serious degradations especially for temperature sensitive drug. However the use of high energy radiation such as gamma rays for sterilization offers clear advantages but radiosensitivity of irradiated pharmaceuticals is an important aspect. This potential of radiosensitivity made Sultamicillin to be used as dosimetric material and it was investigated by electron spin resonance spectroscopy.

11.Schutz.,[41](2009) detailed the efficacy and safety of Sultamicillin in an open randomized multicentric study of two aminopenicillin/β-lactamase inhibitor combinations for oral administration ie; a comparision between Sultamicillin (750 mg bid) and amoxicillin/clavulanate (625 mg tid) in 132 patients with uncomplicated urinary tract infections. Both combinations were well tolerated and troublesome adverse effects were not seen. The patients were divided into two groups and one received Sultamicillin and the other a combination of amoxyillin and clavulanate. In the Sultamicillin group 95.3% were treated successfully whereas in amoxicillin/clavulanate it was 90.3%; thus proving sultamicillin more efficacious. Moreover Sultamicillin has the advantage of only twice-daily dosing. between 0.1-1.1 mg/l.

12. V J Kumar, P B Gupta *et al*[42]**.,(2011)**found a new degradant substance during gradient reverse phase HPLC analysis of stability storage samples. This method is also used for identification, isolation and characterization of the new degradant. The level of the degradant impurity was observed up to 1.0%. The impurity was identified by LC –MS and was characterized by H- NMR, C-NMR, 2D-NMR, LC/MS/MS, MS/TOF, elemental analysis and IR. The impurity identified was formaldehyde adduct with 5-oxo-4-phenylimidazolidin-1-yl moiety which was later prepared by isolation and co-injected into column to confirm retention time.

Aim and plan of work

AIM AND PLAN OF WORK

AIM

To develop and validate a new isocratic RP-HPLC and HPTLC methods for the determination of sultamcillin tosylate in tablet dosage form.

To validate the method with respect to linearity, precision, accuracy and robustness.

To develop the method suitable for routine analysis of sultamcillin tosylate in pharmaceutical formulation.

OBJECTIVE

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose.

PLAN OF WORK

1. Solubility data,analytical data is to be studied to develop the initial conditions.
2. Selection of initial separation conditions and trails for assay of sultamcillin tosylate in pharmaceutical dosage form.
3. To develop a method for the assay of sultamcillin tosylate in pure and marketed sample by RP-HPLC and HPTLC.

To validate the developed assay method according to ICH method validation parameters such as system suitability, linearity, precision, accuracy, robustness,etc

Materials and Methods for Hplc

MATERIALS AND INSTRUMENTS

MATERIALS AND METHODS

1) Balance (Ascoset, model ER200A)
2) pH meter(ADWA,modelAD1020)
3) HPLC- Shimadzu prominence2010
4) Detector-UV-VIS detector
5) Chromatographic data software-spectrum ES
6) Column- kromasil100 ,C-18 (4.6mmx 3.5µ)

Reagents and chemicals

1) Methanol (HPLC grade)-Merck
2) Purified water(HPLC grade)- Millipore
3) Ortho phosphoric acid(AR grade)- Rankem

4)Sodiumdihydrogenortho phosphate dehydrate (AR garde)

5) Acetonitrile (HPLC grade)

SULTAMCILLIN TOSYLATE- Working standard

SULTAMCILLIN TABLET- 500mg

METHOD DEVELOPMENT AND OPTIMIZATION

Chromatographic conditions

Table 5

Instrument	Shimadzu 2010 prominence
Column	Kromasil C18,3.5µ(100mm×4.6mm)
Buffer	phosphate buffer solution pH 3
Flow rate	1.0ml/min.
Wavelength	215 nm
Injection volume	10µl
Column temperature	25
Runtime	20minutes
Mobile phase	Acetonitrile: phosphate buffer(20:80)
Elution technique	isocratic

MOBILE PHASE PREPARATION:-

Acetonitrile hplc grade

Phosphate buffer solution:-

About 4.68 g of Sodium dihydrogenortho phosphate dihydrate was dissolved in 1 litre of water and sonicated for a period of 5 minutes and then pH of the solution was adjusted to 3 ± 0.05 using orthophosphoric acid. The buffer was filtered through a 0.45 µm membrane filter.

The ratio of mobile phase is (buffer) 80: (Acetonitrile)20.

Diluent:

Mobile phase is used as diluent.

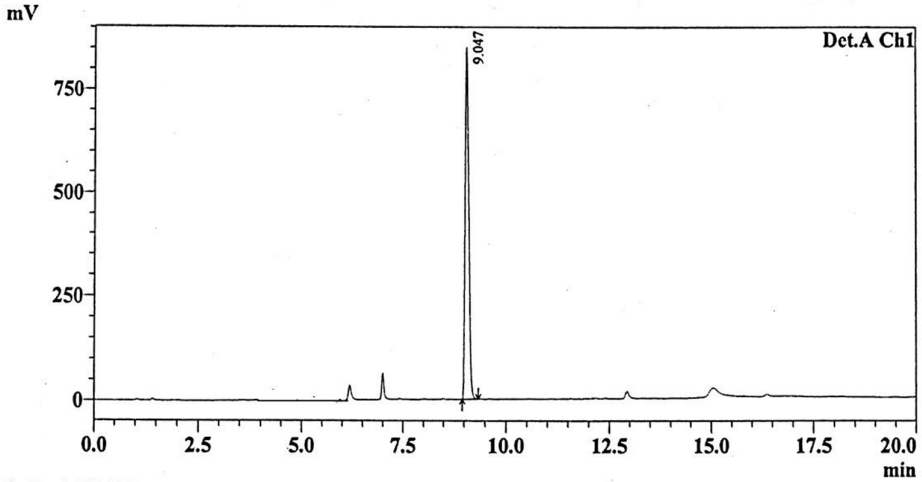
Standard preparation

Accurately weighed 50mg of sultamcillin tosylate WS in 100 ml volumetric flask and 50 ml of diluent was added. Sonicated to dissolve and made up the volume with diluent. Pipetted out 5ml of above solution in 100ml volumetric flask and make up the volume with diluent.

Sample preparation

Weighed 20 tablets crush and accurately weighed100mg equ. of sultamcillin tosylate in 200ml volumetric flask and100ml of diluent was added,sonicated for 20 minutes and make up the volume with diluent. Pipetted 5ml ofabove solution in 100ml volumetric flask and make up the volume with diluent.

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Standard
Tray#	: 1
Vail#	: 6
Injection Volume	: 10 uL
Data Filename	: QCLC003 27101014.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Assay of sultamcillin Tosilate



1 Det.A Ch1 / 215nm

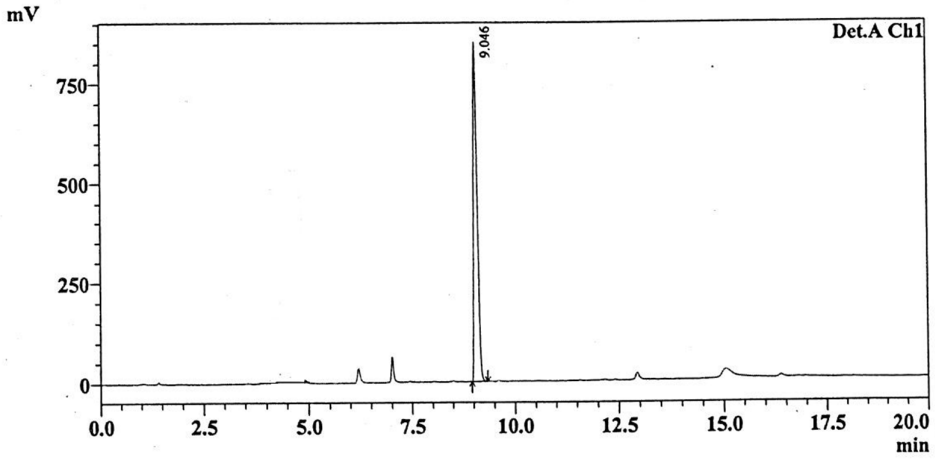
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.05	5345342	100.00	Sultamicillin
Total		5345342	100.00	

CHROMATOGRAM-1

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Sample
Tray#	: 1
Vail#	: 7
Injection Volume	: 10 uL
Data Filename	: QCLC003 27101016.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Assay of sultamcillin Tosilate



1 Det.A Ch1 / 215nm

PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.05	5372840	100.00	Sultamicillin
Total		5372840	100.00	

CHROMATOGRAM-2

Assay % =

$$\frac{AS}{AStd} \times \frac{Wt.std}{Dil.std} \times \frac{Dil.s}{Wt.s} \times \frac{\%.std}{100} \times \frac{Avg.\ Wt.}{LC} \times 100$$

Where: As = average area of sample preparation.

Astd= average area of standard preparation.

Wt.Std = Weight of working standard taken in mg.

%std = Percentage purity of working standard

LC =Label claim of sultamcillin tosilate

Wt.s= weight of sample

Dil.s = dilution factor of sample

Dil.std = dilution factor of standard

Validation of the analytical method

The validation protocol is applicable to quantitative analytical method – assay, which is used for the estimation of active content in support of regulatory requirements. This procedure covers the following validation parameters.

1)System Suitability

2)Specificity

3)Linearity and

4)Accuracy

5)Precision

System precision/Reproducibility

Method Precision/ Repeatability

6)Robustness

7)Solution stability

SYSTEM SUITABILITY:-

Standard Preparation

Weighed accurately 50mg of sultamcillin tosilate WS in 100 ml volumetric flask and 50 ml of diluent was added and sonicate to dissolve and make up the volume with diluent. Pipetted 5ml of above solution in 100ml volumetric flask and make up the volume with diluent.

Procedure

Injected 10µl of sultamcillin tosilate in replicate (5 times) and recorded the peak area response of sultamcillin tosilate. Calculated the % RSD for the response of the standard solution (for 5 replicates).

Table 6 System suitability for sultamcillin tosilate

Injection No.	Response	Theoretical Plate	Tailing Factor
1	5297131	5253	1.10
2	5276193	5264	1.11
3	5304785	5264	1.12
4	5303020	5207	1.10
5	5355219	5253	1.10
Average	5307270	5248.2	1.10
StdDev	29120.11		
RSD	0.54		

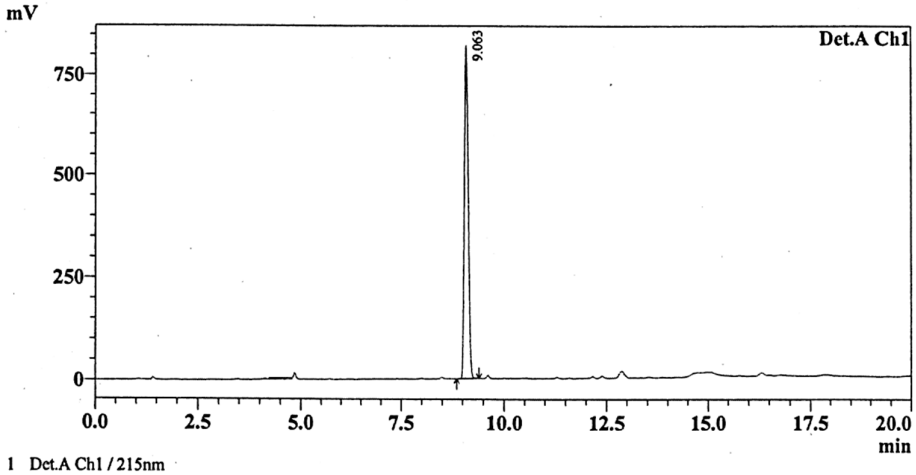
TABLE 7

Parameter	Sultamcillin
Retention time(min.)	9.06
Tailing Factor	1.10
Number of Theoretical Plates	5248.2

Acceptance criteria

The % RSD should not be more than 2.0%, USP tailing should not be more than 2.5, and Theoretical plates should not be less than 1500.

Instrument ID.No.	**: QCLC-003**
Sample Name	**: Sultamicillin Tosilate**
Sample ID	**: System Suitability-1**
Tray#	**: 1**
Vail#	**: 30**
Injection Volume	**: 10 uL**
Data Filename	**: QCLC003 29101019.lcd**
Method Filename	**: Sulatamicillin Tosilate (EP).lcm**
Batch Filename	**: STM Tosilate.lcb**
Description	**: Analytical Method Validation-Sultamicillin Tosilate**



1 Det.A Ch1 / 215nm

PeakTable

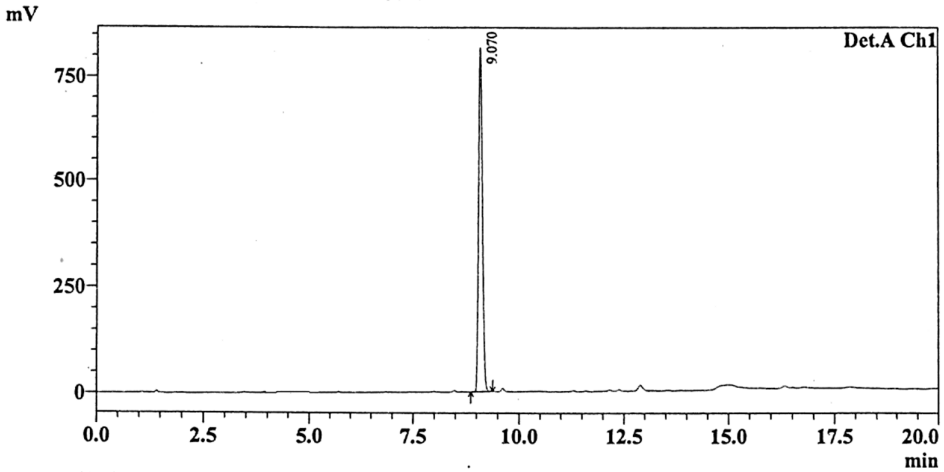
Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.06	5297131	100.00	Sultamicillin
Total		5297131	100.00	

Theoretical plates	Tailing factor
5253	1.1

CHROMATOGRAM-3

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: System suitability-2
Tray#	: 1
Vail#	: 29
Injection Volume	: 10 uL
Data Filename	: QCLC003 29101018.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

PeakTable

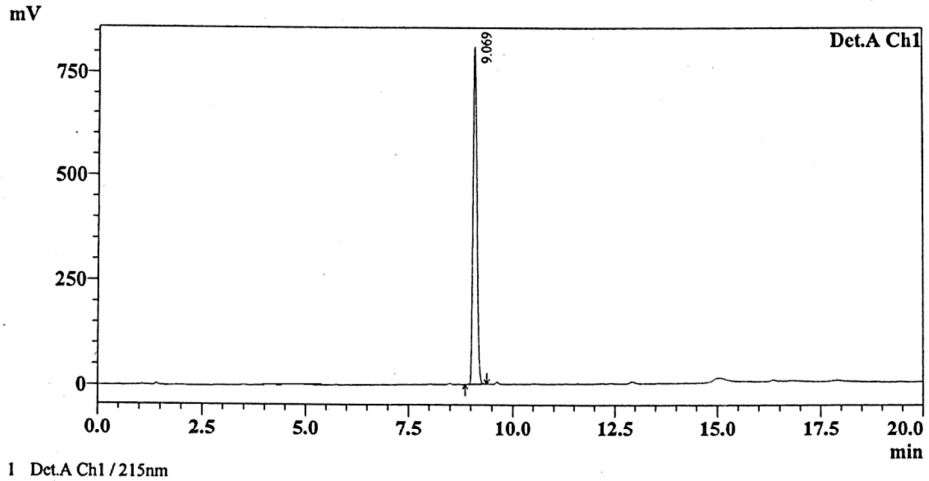
Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.07	5276193	100.00	Sultamicillin
Total		5276193	100.00	

Theoretical plates	Tailing factor
5264	1.10

CHROMATOGRAM-4

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	:System suitability-3
Tray#	: 1
Vail#	: 23
Injection Volume	: 10 uL
Data Filename	: QCLC003 29101012.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

PeakTable

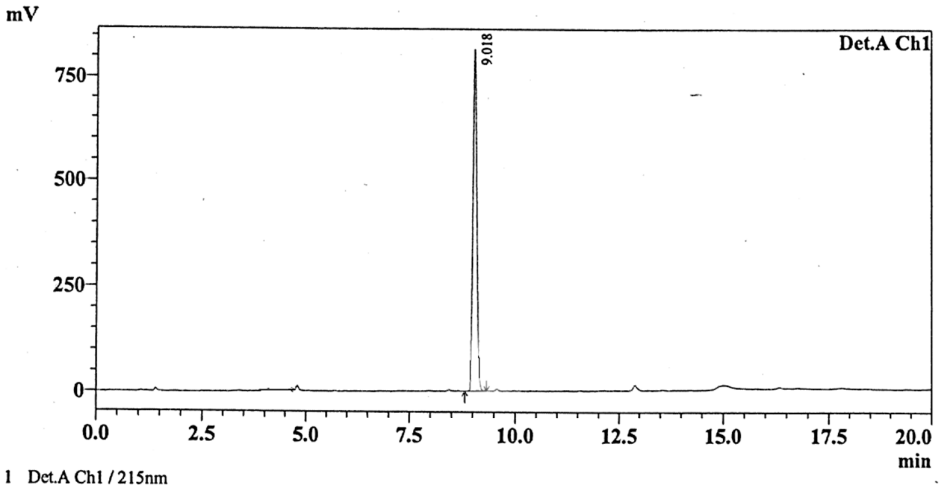
Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.07	5304785	100.00	Sultamicillin
Total		5304785	100.00	

Theoretical plates	Tailing factor
5264	1.12

CHROMATOGRAM-5

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: System suitability- 4
Tray#	: 1
Vail#	: 21
Injection Volume	: 10 uL
Data Filename	: QCLC003 29101010.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

PeakTable

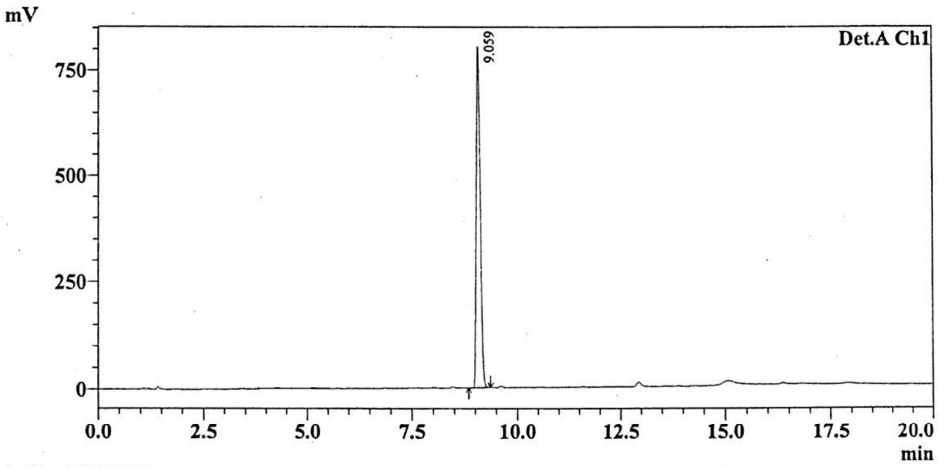
Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.02	5303020	100.00	Sultamicillin
Total		5303020	100.00	

Theoretical plates	Tailing factor
5207	1.0

CHROMATOGRAM-6

Instrument ID.No.	**: QCLC-003**
Sample Name	**: Sultamicillin Tosilate**
Sample ID	**: System suitability-5**
Tray#	**: 1**
Vail#	**: 17**
Injection Volume	**: 10 uL**
Data Filename	**: QCLC003 29101006.lcd**
Method Filename	**: Sulatamicillin Tosilate (EP).lcm**
Batch Filename	**: STM Tosilate.lcb**
Description	**: Analytical Method Validation-Sultamicillin Tosilate**



1 Det.A Ch1 / 215nm

PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.06	5355219	100.00	Sultamicillin
Total		5355219	100.00	

Theoretical plates	Tailing factor
5253	1.1

CHROMATOGRAM-7

SPECIFICITY

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present such as the impurities, degradation products and matrix components..

Preparation of standard solution

Weighed accurately 50mg of sultamcillin tosilateWS in 100 ml volumetric flask and 50 ml of diluent was added and sonicated to dissolve and make up the volume with diluent. Pipetted 5ml of above solution in 100ml volumetric flask and make up the volume with diluent.

Preparation of sample solution

Weighed 20 tablet crush, weighed accurately 100mg equ. of sultamcillin tosilate in 200ml volumetric flask and added 100ml of diluent sonicate for 20 minutes and make up the volume with diluent. Pipette 5ml of above solution in 100ml volumetric flask and make up the volume with diluent.

Preparation of placebo solution

Weighed 124 mg of placebo into 100 ml flask, add 50 ml of diluent sonicate and make up the volume with diluent. Pipetted 5ml of above solution in 100ml volumetric flask and make up the volume with diluent

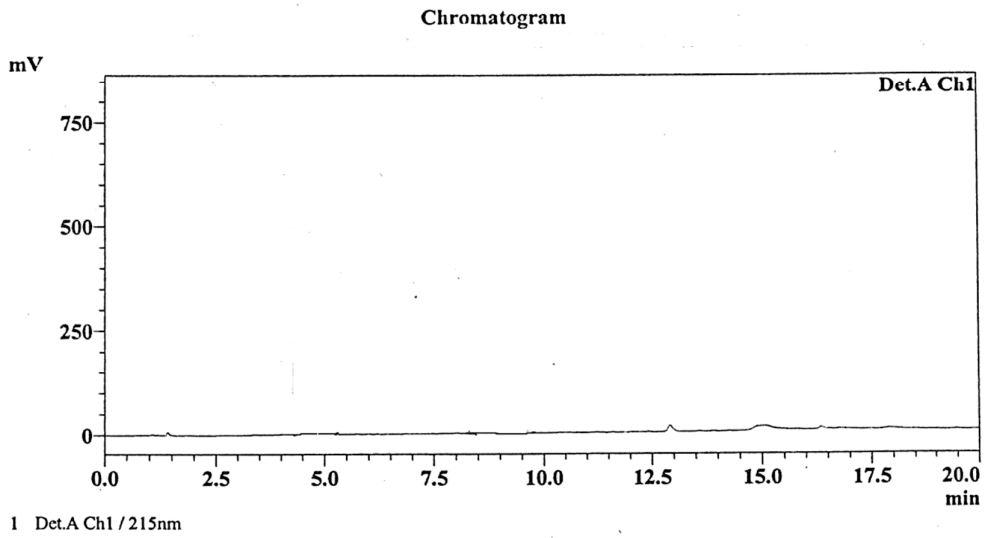
Procedure

The sample is injected in the order Blank, Standard, Sample, and recorded the chromatograms.The data shall be evaluated for any interference from the blank and placebo.

Acceptance criteria There should not be any interference due to placebo in sample and standard preparation.

ConclusionArea due to placebo is Nil. Hence complies.

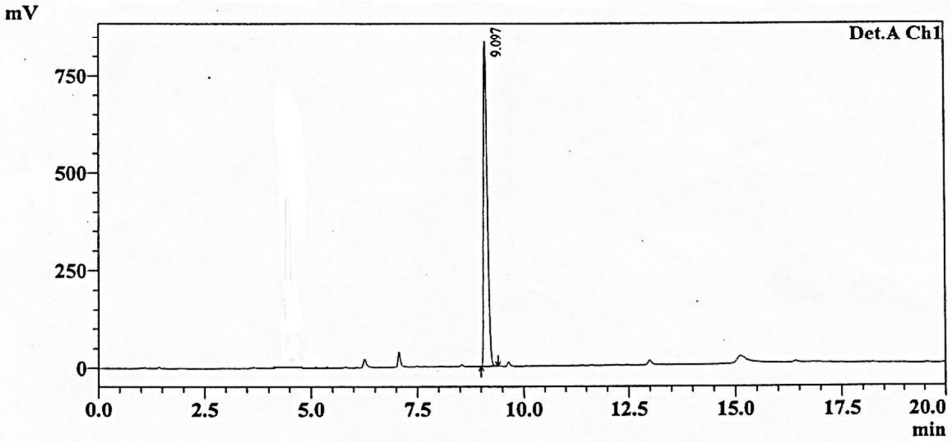
Instrument ID.No.	**: QCLC-003**
Sample Name	**: Sultamicillin Tosilate**
Sample ID	**: Blank**
Tray#	**: 1**
Vail#	**: 12**
Injection Volume	**: 10 uL**
Data Filename	**: QCLC003 29101001.lcd**
Method Filename	**: Sulatamicillin Tosilate (EP).lcm**
Batch Filename	**: STM.lcb**
Description	**: Analytical Method Validation-Sultamicillin Tosilate**



CHROMATOGRAM-8

Instrument ID.No.	**: QCLC-003**
Sample Name	**: Sultamicillin Tosilate**
Sample ID	**: Specificity Standard**
Tray#	**: 1**
Vail#	**: 12**
Injection Volume	**: 10 uL**
Data Filename	**: QCLC003 27101025.lcd**
Method Filename	**: Sulatamicillin Tosilate (EP).lcm**
Batch Filename	**: STM Tosilate.lcb**
Description	**: Analytical Method Validation-Sultamicillin Tosilate**

Chromatogram



1 Det.A Ch1 / 215nm

PeakTable

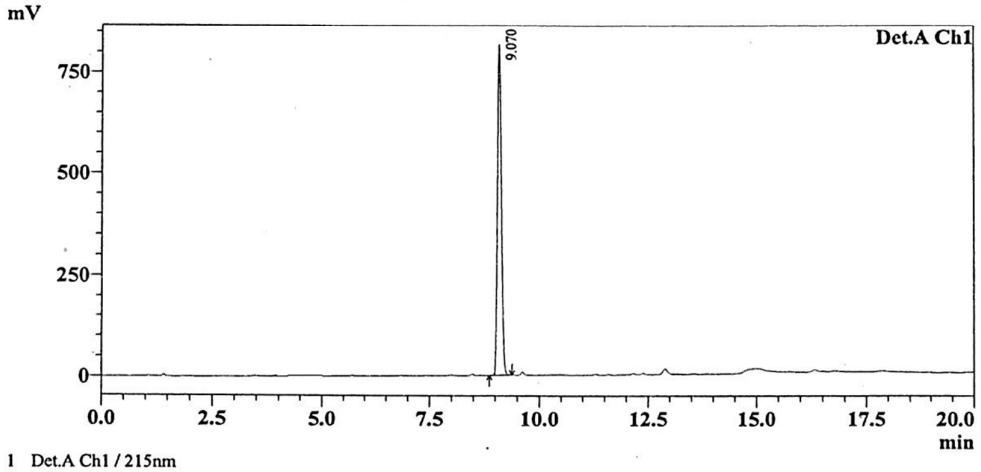
Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.10	5171421	100.00	Sultamicillin
Total		5171421	100.00	

CHROMATOGRAM-9

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Specificity-sample
Tray#	: 1
Vail#	: 29
Injection Volume	: 10 uL
Data Filename	: QCLC003 29101018.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate

Chromatogram



1 Det.A Ch1 / 215nm

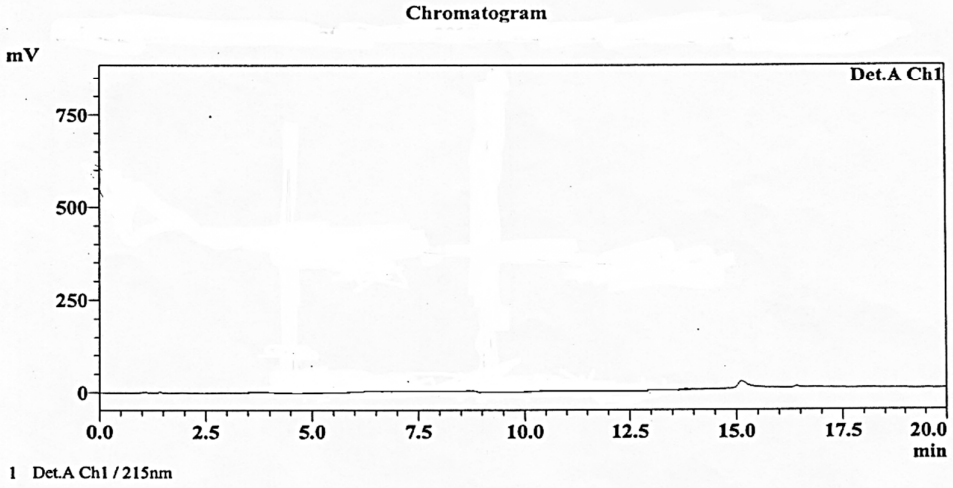
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.07	5276193	100.00	Sultamicillin
Total		5276193	100.00	

CHROMATOGRAM-10

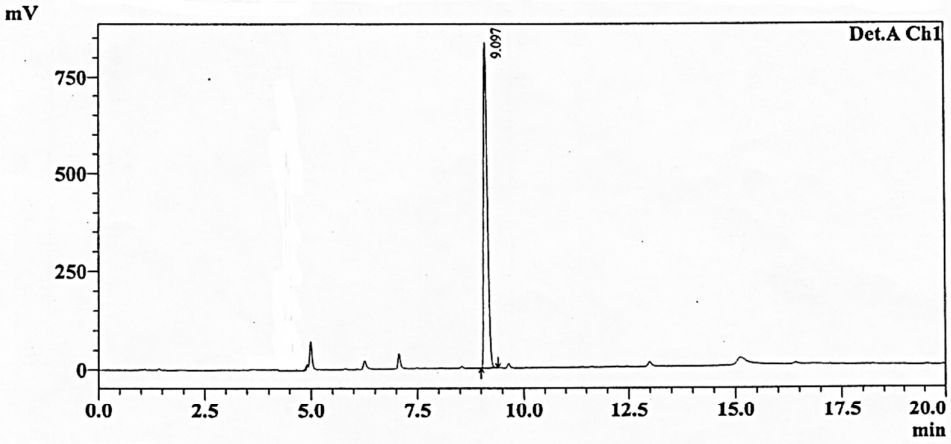
Instrument ID.No.	**: QCLC-003**
Sample Name	**: Sultamicillin Tosilate**
Sample ID	**: Specificity Placebo**
Tray#	**: 1**
Vail#	**: 12**
Injection Volume	**: 10 uL**
Data Filename	**: QCLC003 27101025.lcd**
Method Filename	**: Sulatamicillin Tosilate (EP).lcm**
Batch Filename	**: STM Tosilate.lcb**
Description	**: Analytical Method Validation-Sultamicillin Tosilate**



CHROMATOGRAM-11

Instrument ID.No.	**: QCLC-003**
Sample Name	**: Sultamicillin Tosilate**
Sample ID	**: Specificity Standard+Placebo**
Tray#	**: 1**
Vail#	**: 12**
Injection Volume	**: 10 uL**
Data Filename	**: QCLC003 27101025.lcd**
Method Filename	**: Sulatamicillin Tosilate (EP).lcm**
Batch Filename	**: STM Tosilate.lcb**
Description	**: Analytical Method Validation-Sultamicillin Tosilate**

Chromatogram



1 Det.A Ch1 / 215nm

PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.10	5171421	100.00	Sultamicillin
Total		5171421	100.00	

CHROMATOGRAM -12

Linearity

The linearity of an analytical procedure is its ability to elicit test results that are directly, or by a well – defined mathematical transformation, proportional to the concentration of analyte in samples within a given range.

Preparation of linearity stock solute

Weighed accurately 50 mg of sultamcillin tosylate WS into 200ml flask, Dissolved and make up the volume with diluent.

Level 1 solution (50 %) Pipetted out 5 ml of the linearity stock solution into a 100ml standard flask and make up the volume with diluent.

Level 2 solution (75 %)

Pipetted out 15 ml of the linearity stock solution into a 200ml ml standard flask and make up the volume with diluent

Level 3 solution (100 %)

Pipetted out 5 ml of the linearity stock solution into a 50ml standard flask and make up the volume with diluent

Level 4 solution (125 %)

Pipetted out 25 ml of the linearity stock solution into a 200ml standard flask and make up the volume with diluent

Level 5 solution (150 %)

Pipetted out 15 ml of the linearity stock solution into a 100ml ml standard flask make uptothe volume with diluent.

Plot the linearity curve of the standard solution response against the respective concentration.

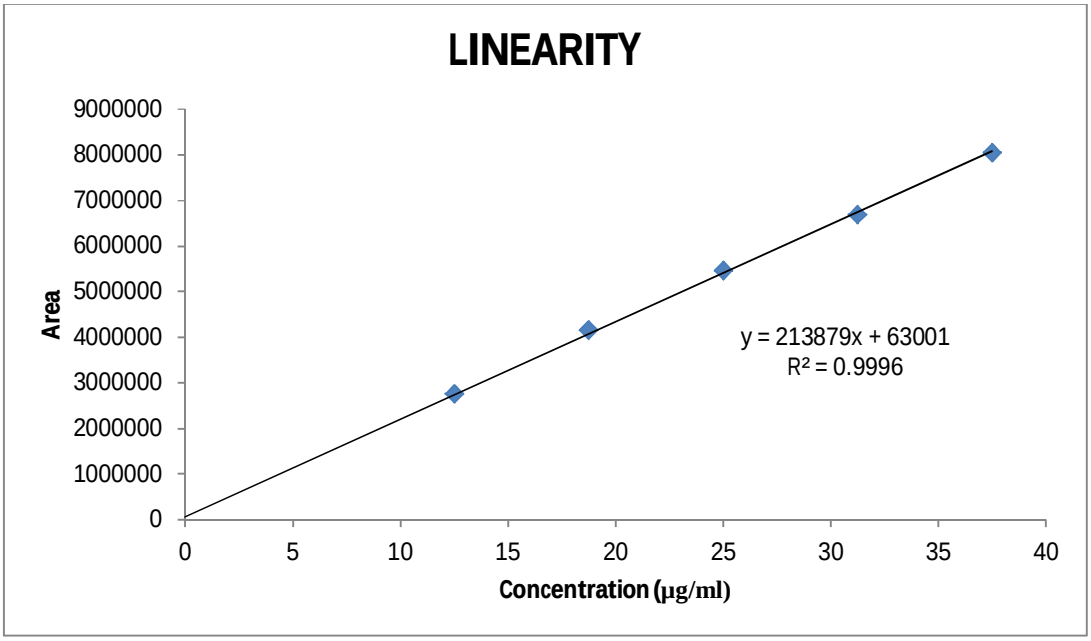
Table 8 Linearity concentration range

Linearity Solution	Working Concentration (mcg/ml)
Level 1	12.50
Level 2	18.75
Level 3	25.00
Level 4	31.25
Level 5	27.50

Table-9

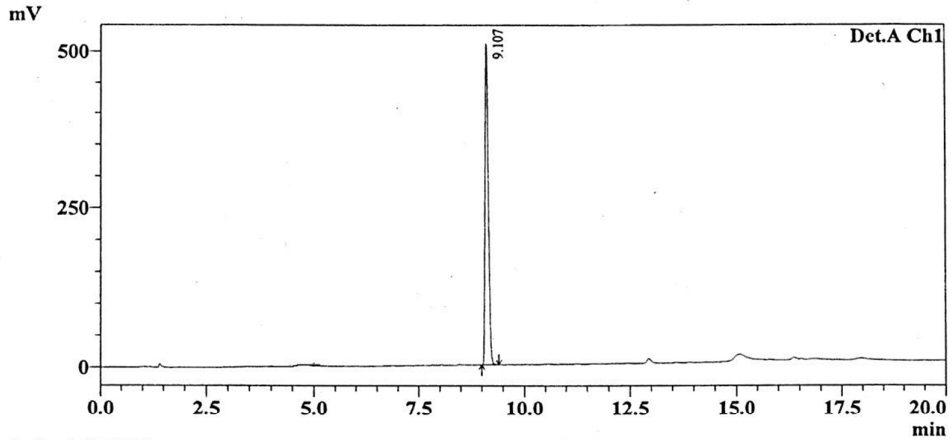
%	Con(mcg/ml)	Average Response
50	12.5	2761181
75	18.75	4148018
100	25	5466784
125	31.25	6688020
150	37.5	8048904

FIG-8



Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Linearity-[50%]-1
Tray#	: 1
Vail#	: 5
Injection Volume	: 10 uL
Data Filename	: QCLC003 28101019.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate

Chromatogram



1 Det.A Ch1 / 215nm

PeakTable

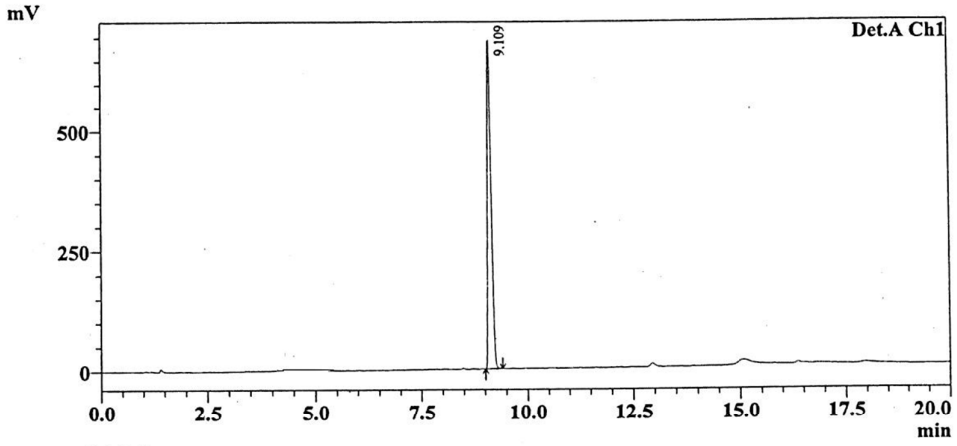
Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.11	2724934	100.00	Sultamicillin
Total		2724934	100.00	

CHROMATOGRAM-13

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Linearity-[75%]-2
Tray#	: 1
Vail#	: 6
Injection Volume	: 10 uL
Data Filename	: QCLC003 28101021.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate

Chromatogram



1 Det.A Ch1 / 215nm

PeakTable

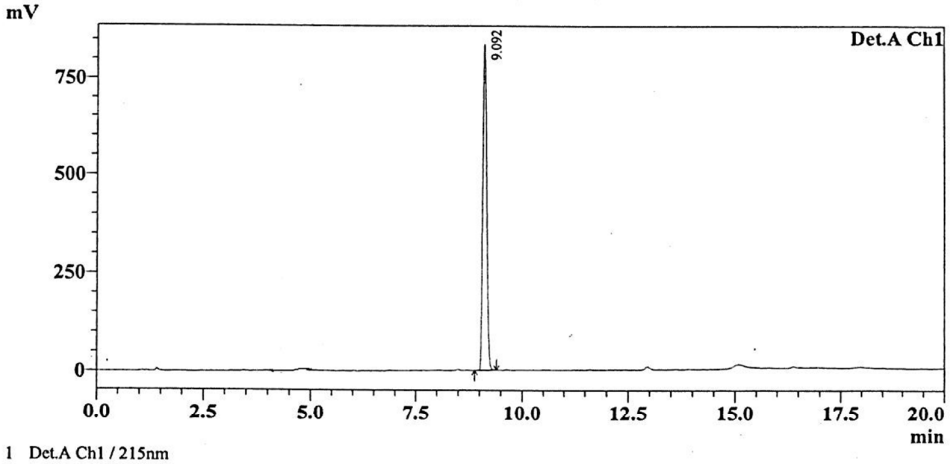
Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.11	4115715	100.00	Sultamicillin
Total		4115715	100.00	

CHROMATOGRAM-14

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Linearity-[100%]- 3
Tray#	: 1
Vail#	: 7
Injection Volume	: 10 uL
Data Filename	: QCLC003 28101024.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate

Chromatogram



1 Det.A Ch1 / 215nm

PeakTable

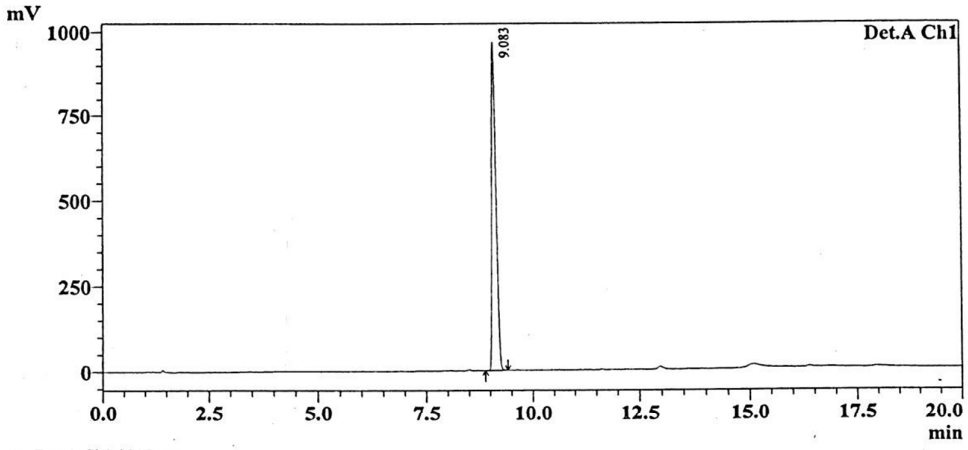
Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.09	5437000	100.00	Sultamicillin
Total		5437000	100.00	

CHROMATOGRAM-15

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Linearity-[125%]-4
Tray#	: 1
Vail#	: 8
Injection Volume	: 10 uL
Data Filename	: QCLC003 28101025.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate

Chromatogram



1 Det.A Ch1 / 215nm

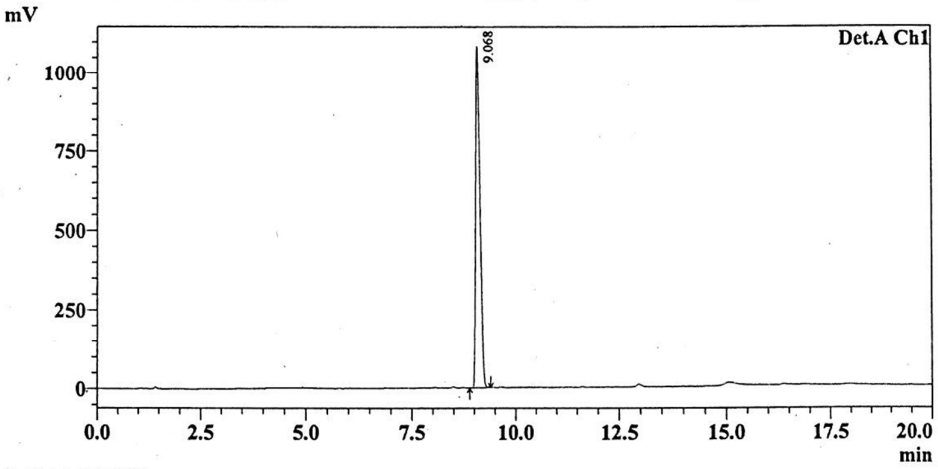
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.08	6717660	100.00	Sultamicillin
Total		6717660	100.00	

CHROMATOGRAM-16

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Linearity-[150%]- 5
Tray#	: 1
Vail#	: 9
Injection Volume	: 10 uL
Data Filename	: QCLC003 28101027.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.07	8021116	100.00	Sultamicillin
Total		8021116	100.00	

CHROMATOGRAM-17

Target Level (%)	Conc. (µg /ml)	Response	Average Response
50	12.5	2724934	2761181
		2843854	
		2714756	
75	18.75	4115715	4148018
		4113625	
		4214714	
100	25	5437000	5466784
		5528142	
		5435210	
125	31.25	6717660	6688620
		6718654	
		6629546	
150	37.5	8021116	8048904
		8022121	
		8103476	
Correlation Coefficient (r^2)			0.9996

Table 10

Acceptance criteria

The linearity regression should not be less than 0.99.

Accuracy:-

The accuracy of an analytical procedure is the closeness of test results obtained by that procedure to the true value. The accuracy of analytical should be established across its range.

Standard Stock Preparation

Weighed accurately 500mg of sultamcillin tosilate WS into 250 ml flask, Dissolved and diluted to volume with diluent. Three standard stock solution as A, B and C was prepared.

Solution A of 80 % concentration

Pipettedout 20 ml of the stock solution and 124 mg of Placebo into a 100 ml standard flask and make up the volume with diluent. Pipettedout 5ml of above solution in 100ml volumetric flask and make up the volume with diluent. Repeat for Solution B and Solution C.

Solution A of 100 % concentration

Pipetted out 25 ml of the stock solution and 124 mg of Placebo into a 100 ml standard flask and make up the volume with diluent. Pipetted out 5ml of above solution in 100ml volumetric flask and make up the volume with diluent. Repeat for Solution B and Solution C.

Solution A of 120 % concentration

Pipettedout 30 ml of the stock solution and 124 mg of Placebo into a 100 ml standard flask and make up the volume with diluent. Pipetted out 5ml of above solution in 100ml volumetric flask and make up the volume with diluent. Repeat for Solution B and Solution C

Procedure

Placebo is prepared based on the manufacturing formula and known concentrations of the standard solutions of 80%, 100% and 120% working concentrations was spiked and estimated for recovery.

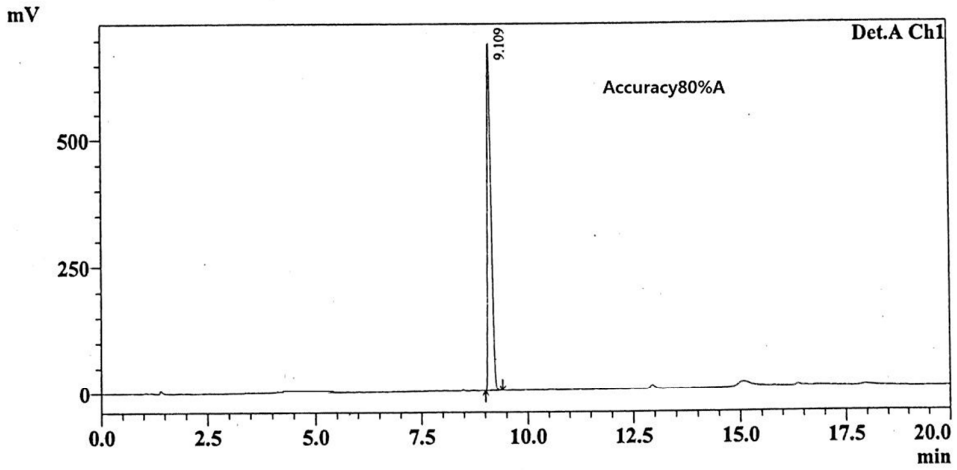
Table11 Accuracy for sultamcillin tosilate

Amount of Known std added with placebo (in %)	Level	Mean area	Recovery (in %)	Average recovery
80%	A	4896129	100.23	99.78
	B	4848960	99.88	
	C	4887599	99.24	
100%	A	5868406	101.44	100.90
	B	5823606	101.12	
	C	5821200	100.13	
120%	A	6748977	101.08	100.41
	B	6745252	99.91	
	C	6665533	100.24	
			Average	100.36

Acceptance criterion

The accuracy should be between 98 – 102%.

Instrument ID.No.	**: QCLC-003**
Sample Name	**: Sultamicillin Tosilate**
Sample ID	**: Accuracy 80%A**
Tray#	**: 1**
Vail#	**: 4**
Injection Volume	**: 10 uL**
Data Filename	**: QCLC003 28101021.lcd**
Method Filename	**: Sulatamicillin Tosilate (EP).lcm**
Batch Filename	**: STM.lcb**
Description	**: Analytical Method Validation-Sultamicillin Tosilate**



1 Det.A Ch1 / 215nm

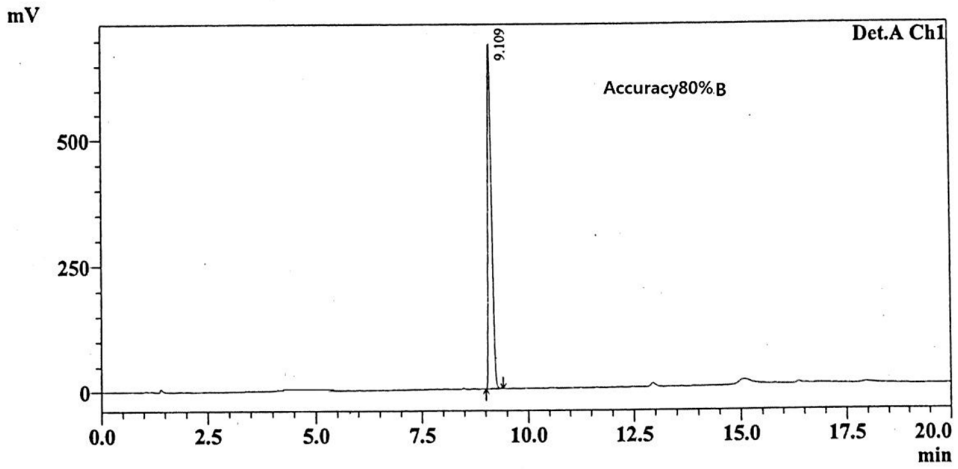
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.11	4896129	100.00	Sultamicillin
Total		4896129	100.00	

CHROMATOGRAM-18

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Accuracy 80%B
Tray#	: 1
Vail#	: 4
Injection Volume	: 10 uL
Data Filename	: QCLC003 28101021.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

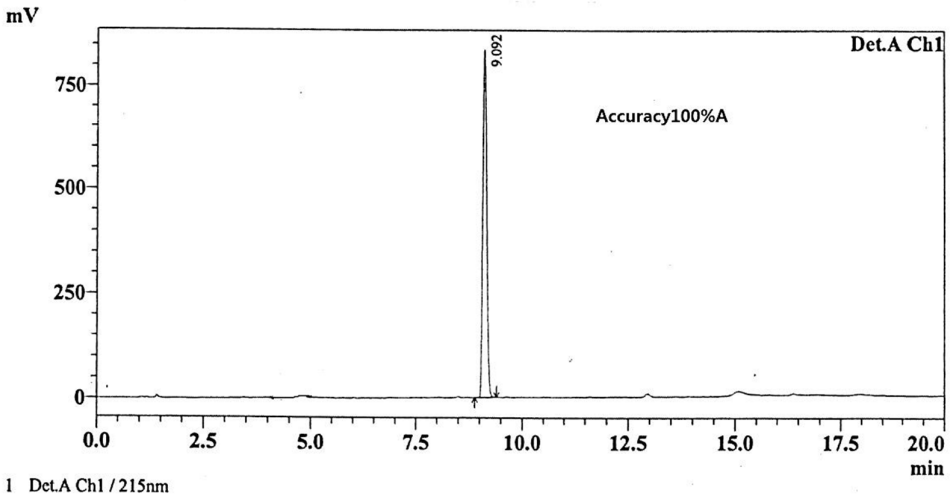
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.11	4848960	100.00	Sultamicillin
Total		4848960	100.00	

CHROMATOGRAM-19

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Accuracy 100%A
Tray#	: 1
Vail#	: 7
Injection Volume	: 10 uL
Data Filename	: QCLC003 28101024.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

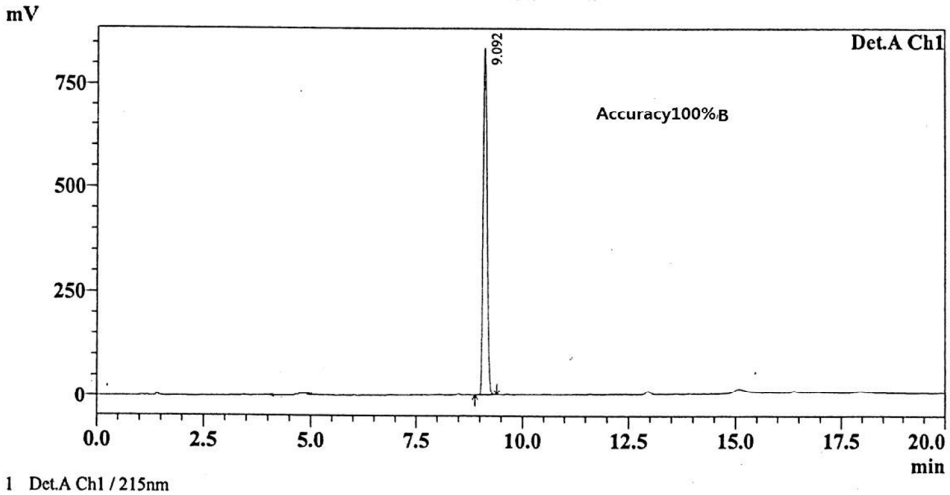
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.09	5868406	100.00	Sultamicillin
Total		5868406	100.00	

CHROMATOGRAM-20

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Accuracy 100%B
Tray#	: 1
Vail#	: 7
Injection Volume	: 10 uL
Data Filename	: QCLC003 28101024.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

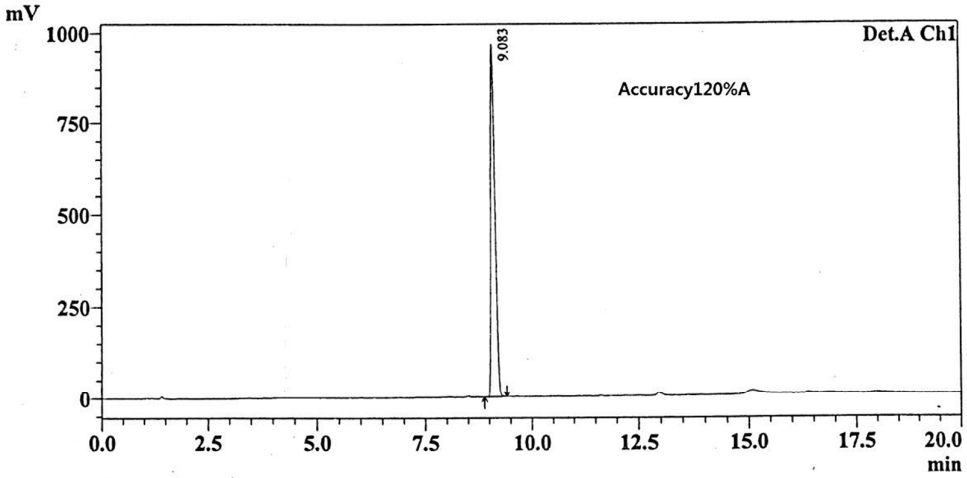
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.09	5823606	100.00	Sultamicillin
Total		5823606	100.00	

CHROMATOGRAM-21

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Accuracy 120%A
Tray#	: 1
Vail#	: 8
Injection Volume	: 10 uL
Data Filename	: QCLC003 28101025.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

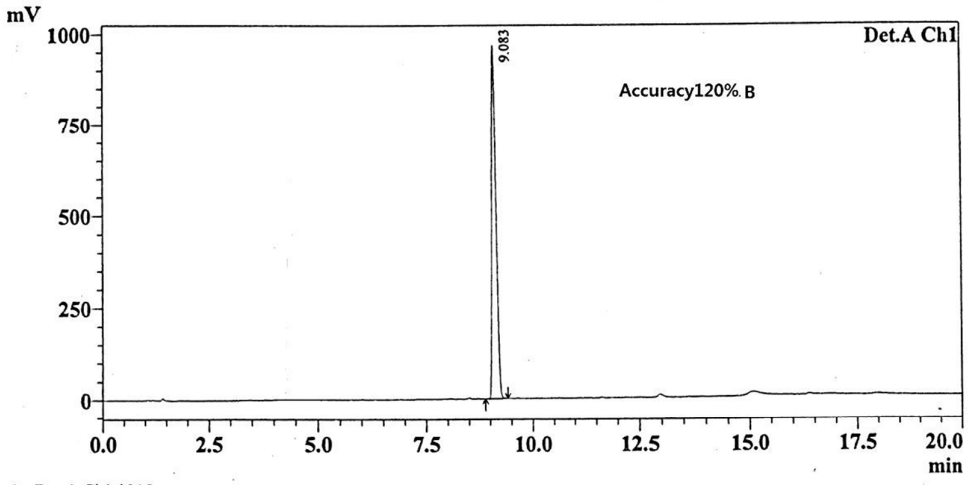
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.08	6748977	100.00	Sultamicillin
Total		6748977	100.00	

CHROMATOGRAM-22

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Accuracy 120% B
Tray#	: 1
Vail#	: 8
Injection Volume	: 10 uL
Data Filename	: QCLC003 28101025.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.08	6754252	100.00	Sultamicillin
Total		6754252	100.00	

CHROMATOGRAM-23

SYSTEM PRECISION

Standard Preparation

Weigh accurately 50mg of sultamcillin WS in 100 ml volumetric flask add 50 ml of diluent sonicate to dissolve and make up the volume with diluent. Pipette 5ml of above solution in 100ml volumetric flask and make up the volume with diluent.

Table.no-12

Sample	Concentration (µg/ml)	Area
1 Standard-injection	100mcg/ml	5202029
2 Standard-injection	100mcg/ml	5199530
3 Standard-injection	100mcg/ml	5194871
4 Standard-injection	100mcg/ml	5205656
5 Standard-injection	100mcg/ml	5208263
6 Standard-injection	100mcg/ml	5182510
	MEAN	5198809.83
	STANDARD DEVIATION	9255.4093
	RSD %	0.17

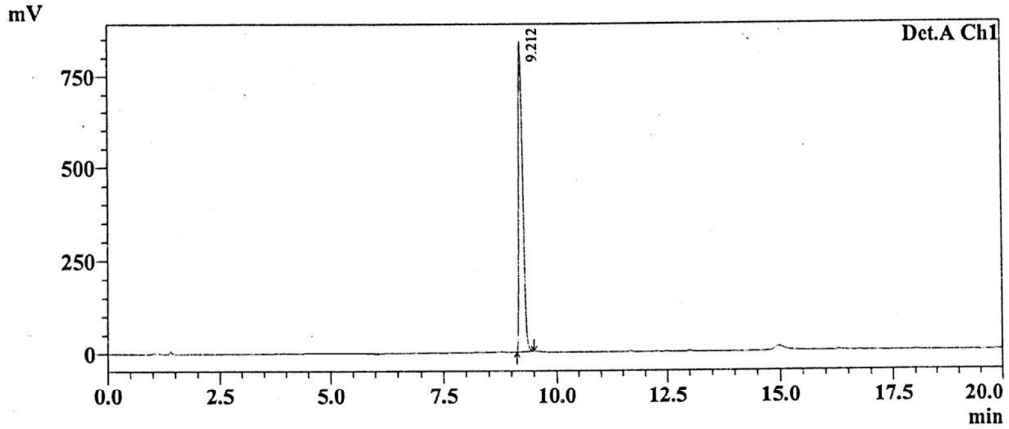
Acceptance criteria

RSD is not more than 2.0%

Conclusion

The precision determined in the above study reveals the reproducibility in the area & the RSD is 0.17 %.Therefore complies.

Instrument ID	**: QCLC-002**
Sample Name	**: Sultamicillin Tosilate**
Sample ID	**: System Precision -1**
Tray#	**: 1**
Vail#	**: 4**
Injection Volume	**: 10 uL**
Data Filename	**: QCLC002 04111004.lcd**
Method Filename	**: STM Tosilate EP Method.lcm**
Batch Filename	**: STM.lcb**
Description	**: Analytical Method Validation**



1 Det.A Ch1 / 215nm

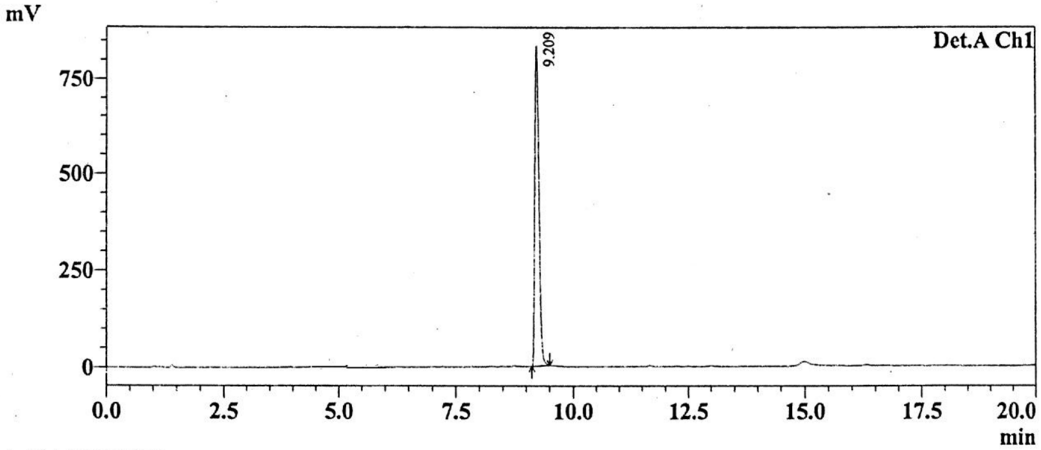
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.21	5202029	100.00	Sultamicillin
Total		5202029	100.00	

CHROMATOGRAM-24

Instrument ID	**: QCLC-002**
Sample Name	**: Sultamicillin Tosilate**
Sample ID	**: System Precision -2**
Tray#	**: 1**
Vail#	**: 4**
Injection Volume	**: 10 uL**
Data Filename	**: QCLC002 04111005.lcd**
Method Filename	**: STM Tosylate EP Method.lcm**
Batch Filename	**: STM.lcb**
Description	**: Analytical Method Validation**



1 Det.A Ch1 / 215nm

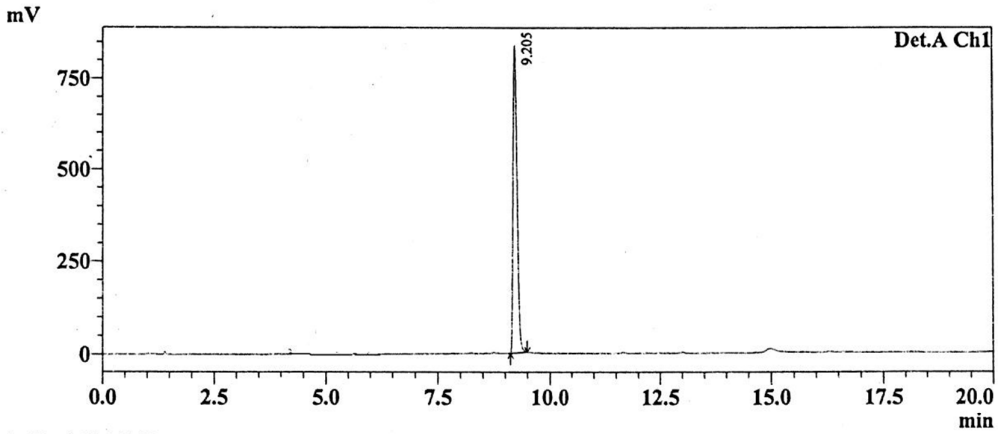
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.21	5199530	100.00	Sultamicillin
Total		5199530	100.00	

CHROMATOGRAM-25

Instrument ID	: QCLC-002
Sample Name	: Sultamicillin Tosilate
Sample ID	: System Precision -3
Tray#	: 1
Vail#	: 4
Injection Volume	: 10 uL
Data Filename	: QCLC002 04111006.lcd
Method Filename	: STM Tosylate EP Method.lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation



1 Det.A Ch1 / 215nm

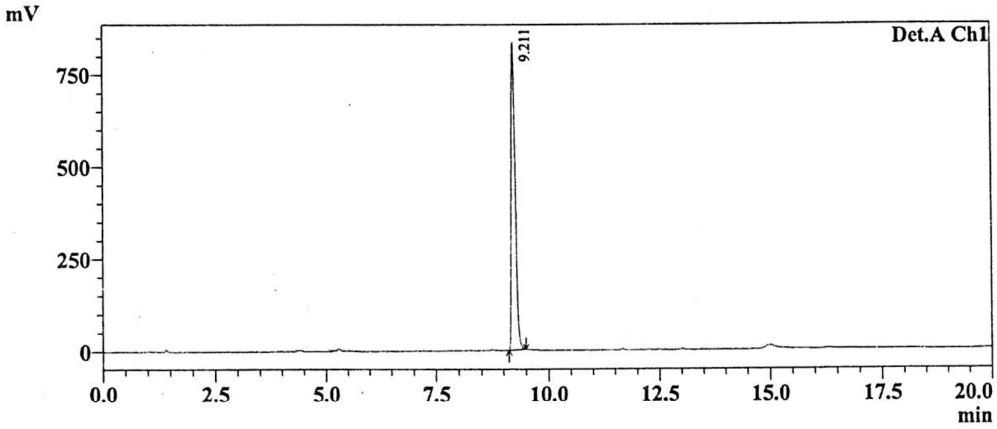
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.20	5194871	100.00	Sultamicillin
Total		5194871	100.00	

CHROMATOGRAM-26

Instrument ID	: QCLC-002
Sample Name	: Sultamicillin Tosilate
Sample ID	: System Precision -4
Tray#	: 1
Vail#	: 4
Injection Volume	: 10 uL
Data Filename	: QCLC002 04111007.lcd
Method Filename	: STM Tosylate EP Method.lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation



1 Det.A Ch1 / 215nm

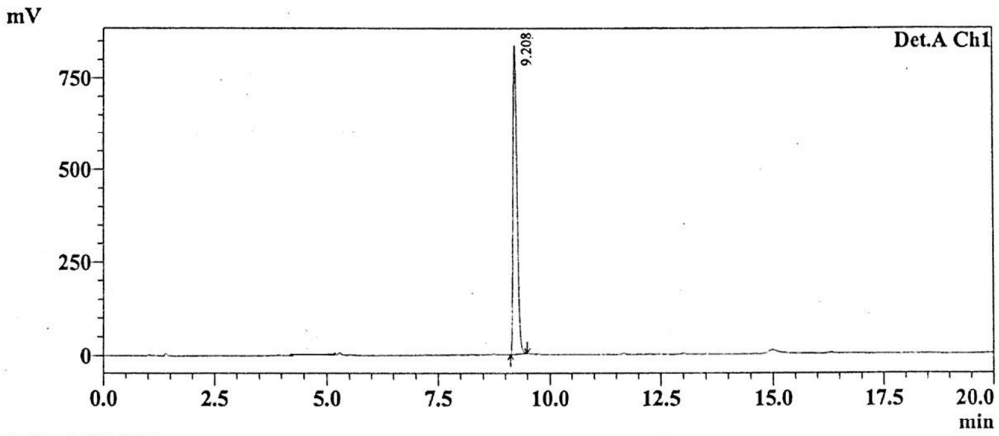
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.21	5205656	100.00	Sultamicillin
Total		5205656	100.00	

CHROMATOGRAM--27

Instrument ID	: QCLC-002
Sample Name	: Sultamicillin Tosilate
Sample ID	: System Precision -5
Tray#	: 1
Vail#	: 4
Injection Volume	: 10 uL
Data Filename	: QCLC002 04111008.lcd
Method Filename	: STM Tosylate EP Method.lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation



1 Det.A Ch1 / 215nm

PeakTable

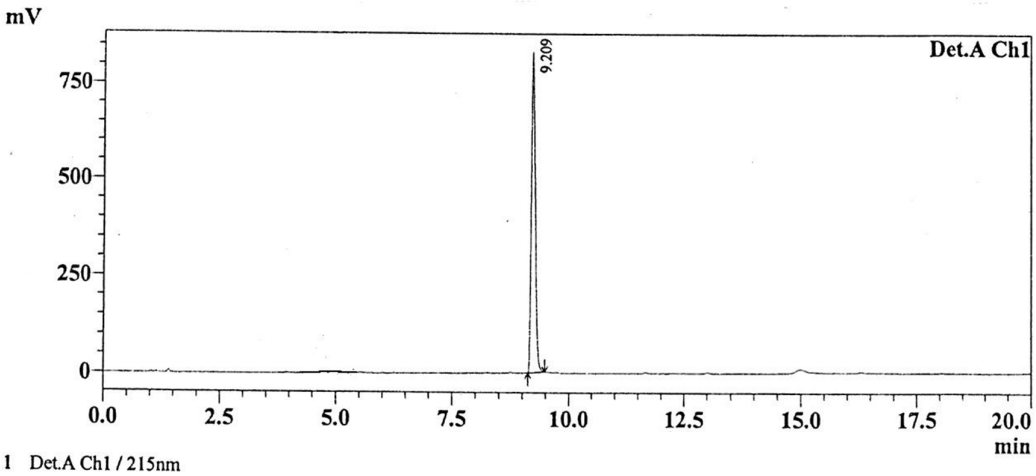
Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.21	5208263	100.00	Sultamicillin
Total		5208263	100.00	

CHROMATOGRAM-28

Instrument ID	: QCLC-002
Sample Name	: Sultamicillin Tosilate
Sample ID	: System Precision -6
Tray#	: 1
Vail#	: 4
Injection Volume	: 10 uL
Data Filename	: QCLC002 04111009.lcd
Method Filename	: STM Tosylate EP Method.lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation

Chromatogram



1 Det.A Ch1 / 215nm

PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.21	5182510	100.00	Sultamicillin
Total		5182510	100.00	

CHROMATOGRAM-29

METHOD PRECISION

Standard Preparation

Weighedaccurately 50mg of sultamcillin tosilate WS in 100 ml volumetric flask add 50ml of diluent sonicate to dissolve and make up the volume with diluent. Pipetted 5ml of above solution in 100ml volumetric flask and make up the volume with diluent.

Sample Preparation

Weighed 20 tablet crush, weighed accurately 100mg equ. of sultamcillin tosilate in 200ml volumetric flask add 100ml of diluent sonicate for 20 minutes and make up the volume with diluent. Pipette 5ml of above solution in 100ml volumetric flask and make up the volume with diluent.

Procedure

Injected 10µl of in replicate (6 times) and recorded the peak area response and calculate. Calculated the assay or recovery value of the sample from each preparation and the % RSD of assay of the sample for all the 6 preparations.

Weight of Sample 1 141.87 mg

Weight of Sample 2 141.90mg

Weight of Sample 3 142.05mg

Weight of Sample 4141.89mg

Weight of Sample 5 141.85 mg

Weight of Sample 6 141.79 mg

Acceptance criterion

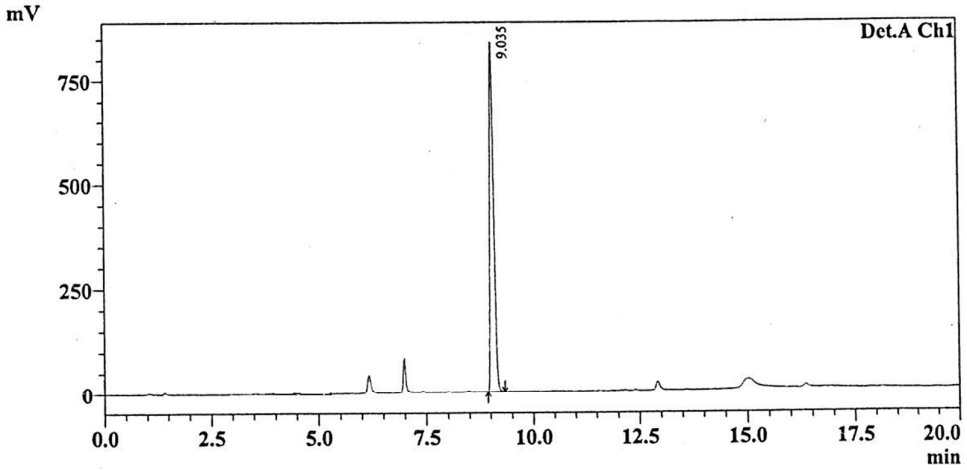
The % RSD of the assay or recovery value of the sultamcillin tosilatefor 6 sample preparations should not be more than 2.00.

Table -13

Method precision for sultamcillin tosilate

Sample No.	Response	Average Response	% Assay
1	5332627	5284199	97.46
	5235771		
2	5343184	5341078	98.48
	5338972		
3	5364140	5410685	99.66
	5457230		
4	5329675	5329320	98.26
	5328965		
5	5345342	5396593.5	99.54
	5447845		
6	5372840	5368380	99.06
	5363920		
		Average	98.74
		StdDev	0.83966
		% RSD	0.8507

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Method Precision-1
Tray#	: 1
Vail#	: 5
Injection Volume	: 10 uL
Data Filename	: QCLC003 27101011.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

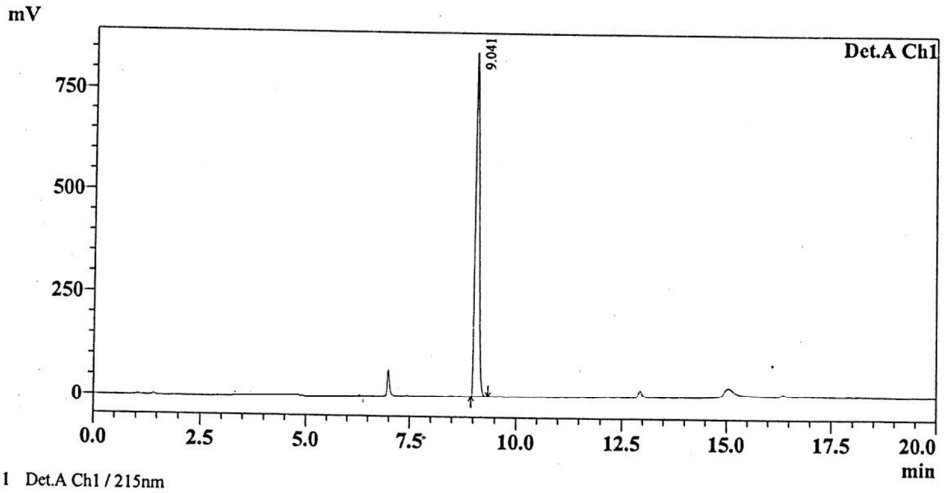
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.03	5332627	100.00	Sultamicillin
Total		5332627	100.00	

CHROMATOGRAM-30

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Method Precision-2
Tray#	: 1
Vail#	: 6
Injection Volume	: 10 uL
Data Filename	: QCLC003 27101013.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

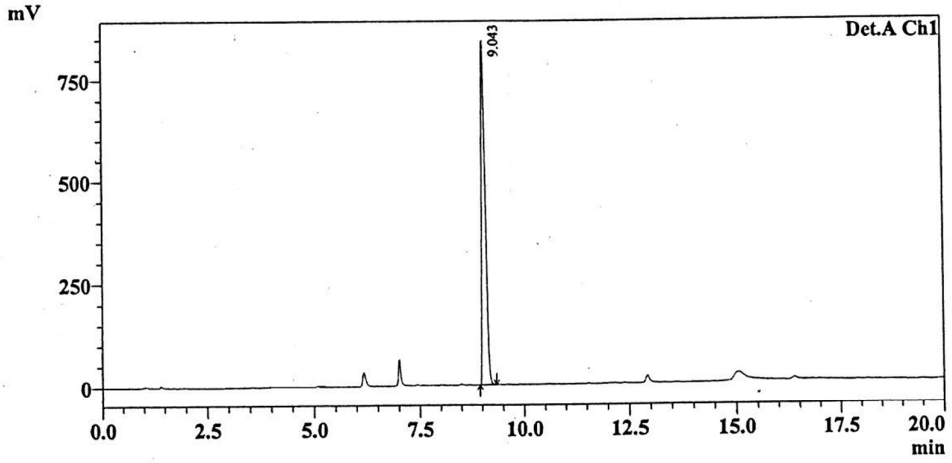
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.04	5343184	100.00	Sultamicillin
Total		5343184	100.00	

CHROMATOGRAM-31

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Method Precision-3
Tray#	: 1
Vail#	: 7
Injection Volume	: 10 uL
Data Filename	: QCLC003 27101015.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

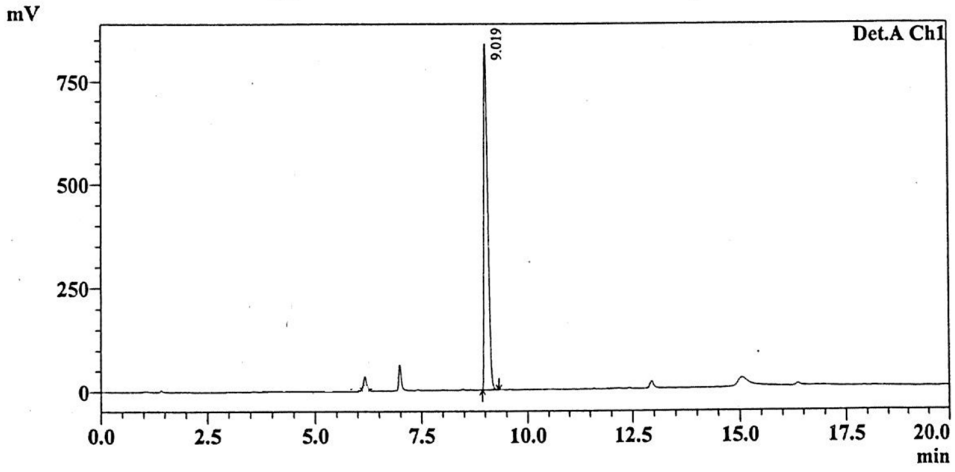
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.04	5364140	100.00	Sultamicillin
Total		5364140	100.00	

CHROMATOGRAM-32

Instrument ID.No.	**: QCLC-003**
Sample Name	**: Sultamicillin Tosilate**
Sample ID	**: Method Precision- 4**
Tray#	**: 1**
Vail#	**: 5**
Injection Volume	**: 10 uL**
Data Filename	**: QCLC003 27101012.lcd**
Method Filename	**: Sulatamicillin Tosilate (EP).lcm**
Batch Filename	**: STM Tosilate.lcb**
Description	**: Analytical Method Validation-Sultamicillin Tosilate**



1 Det.A Ch1 / 215nm

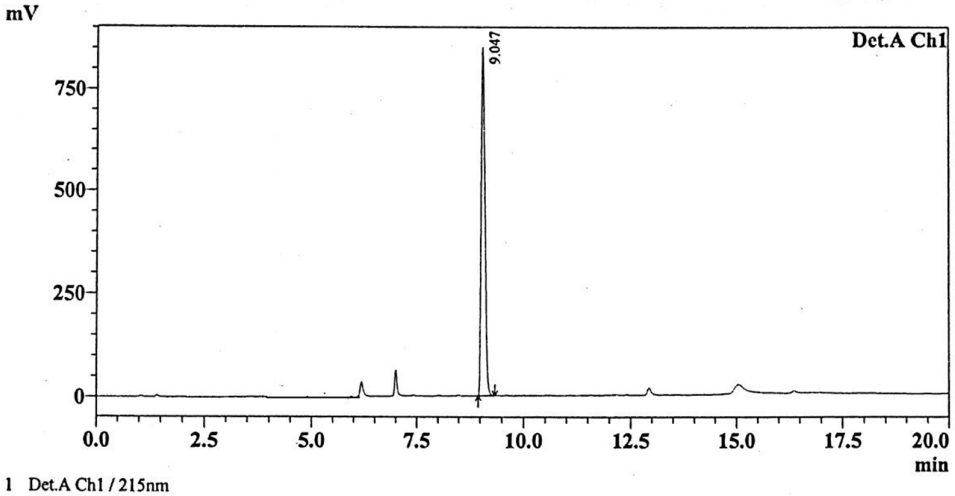
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.02	5329675	100.00	Sultamicillin
Total		5329675	100.00	

CHROMATOGRAM-33

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Method Precision- 5
Tray#	: 1
Vail#	: 6
Injection Volume	: 10 uL
Data Filename	: QCLC003 27101014.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

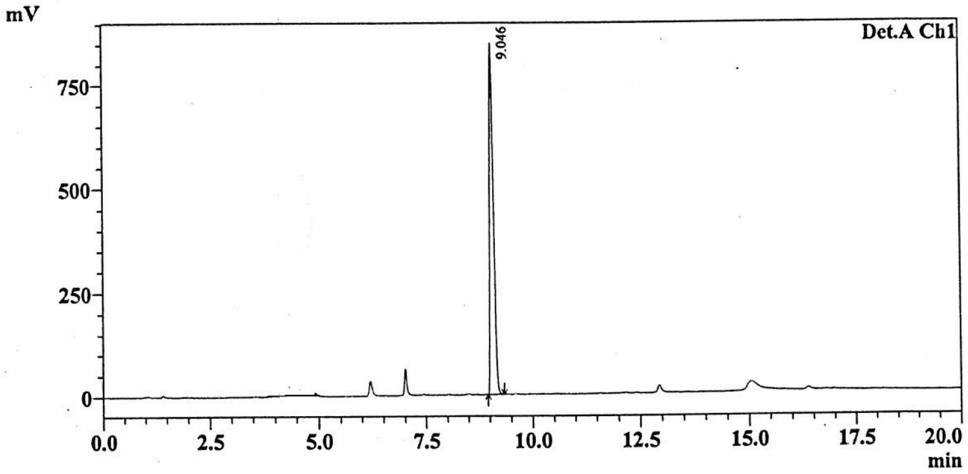
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.05	5345342	100.00	Sultamicillin
Total		5345342	100.00	

CHROMATOGRAM-34

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Method Precision-6
Tray#	: 1
Vail#	: 7
Injection Volume	: 10 uL
Data Filename	: QCLC003 27101016.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.05	5372840	100.00	Sultamicillin
Total		5372840	100.00	

CHROMATOGRAM-35

Robustness

Change of wave length (±2.0 nm)

Six sample preparation of Method precision shall be measured at two different wavelengths using UV-VIS Detector (213 and 217 nm).Calculate the assay or recovery value of the sample from each preparation and the % RSD of assay of the sample for all the 6 preparation

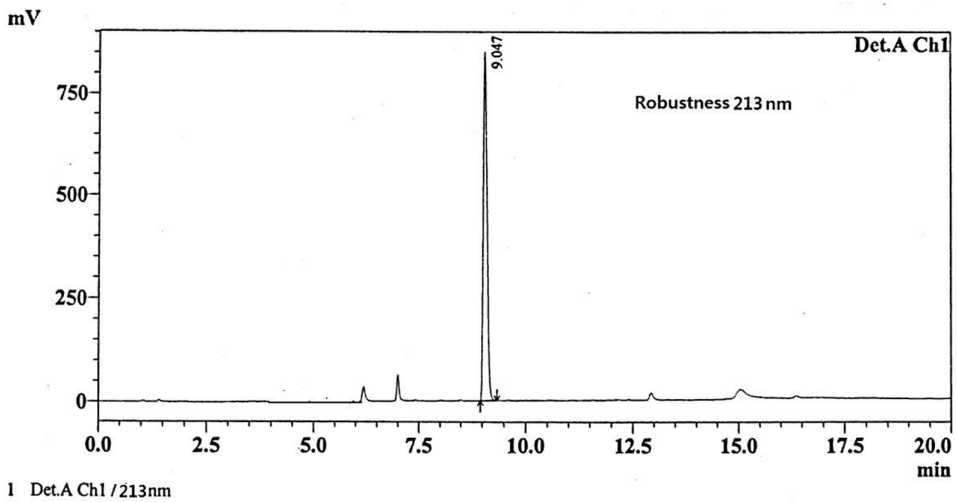
Table 14

Inj	AT 213nm		AT 217nm	
	Response	%Purity	Response	%Purity
1	5328739	98.26	5419405	99.93
2	5344824	100.40	5392517	99.44
3	5307280	98.87	5434544	100.21
4	5412432	99.86	5395316	99.49
5	5354199	98.73	5338899	98.45
6	5374850	99.11	5374850	100.76
Average		99.205		99.623
Std.deviation		0.78701		0.78978
%RSD		0.7933		0.7927

Acceptance criterion

The % RSD should not be more than 5.00.

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Robustness 213nm
Tray#	: 1
Vail#	: 6
Injection Volume	: 10 uL
Data Filename	: QCLC003 27101014.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 213nm

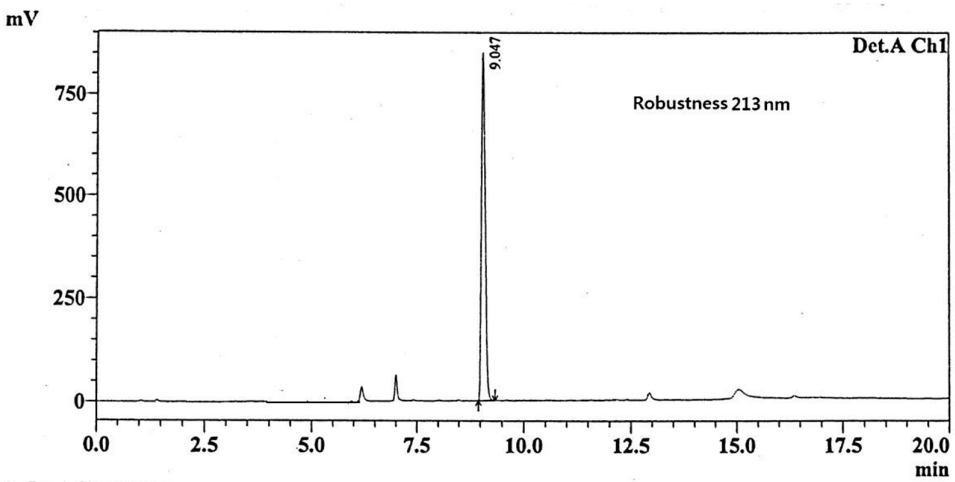
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.05	5328739	100.00	Sultamicillin
Total		5328739	100.00	

CHROMATOGRAM-36

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Robustness 213nm
Tray#	: 1
Vail#	: 7
Injection Volume	: 10 uL
Data Filename	: QCLC003 27101014.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 213nm

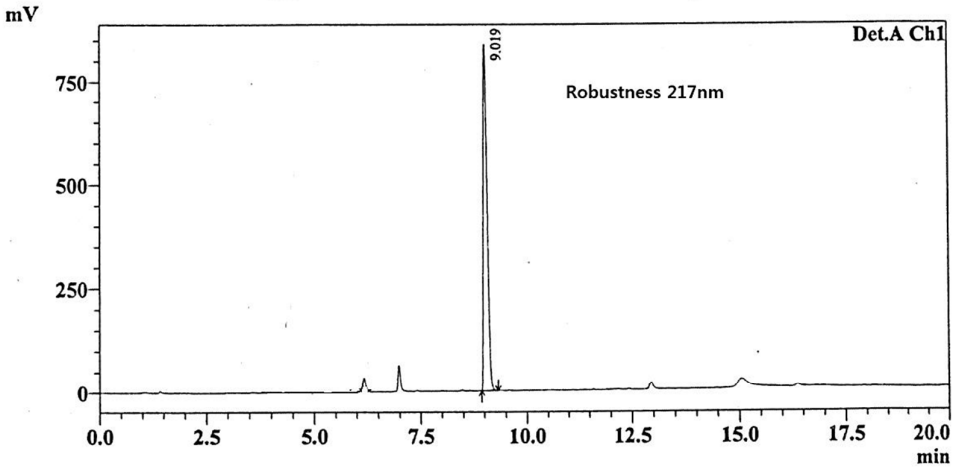
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.05	5412432	100.00	Sultamicillin
Total		5412432	100.00	

CHROMATOGRAM-37

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Robustness 217nm
Tray#	: 1
Vail#	: 5
Injection Volume	: 10 uL
Data Filename	: QCLC003 27101012.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 /217 nm

PeakTable

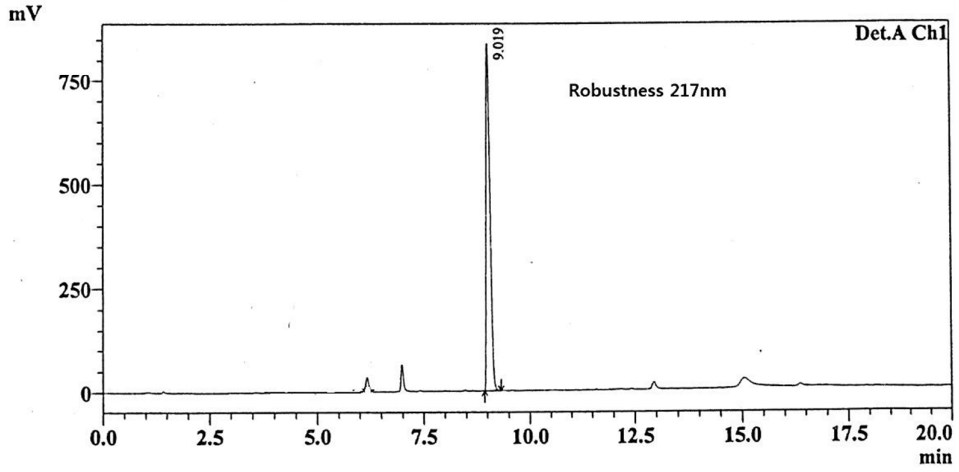
Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.02	5392517	100.00	Sultamicillin
Total		5392517	100.00	

CHROMATOGRAM-38

Instrument ID.No.	**: QCLC-003**
Sample Name	**: Sultamicillin Tosilate**
Sample ID	**: Robustness 217nm**
Tray#	**: 1**
Vail#	**: 4**
Injection Volume	**: 10 uL**
Data Filename	**: QCLC003 27101012.lcd**
Method Filename	**: Sulatamicillin Tosilate (EP).lcm**
Batch Filename	**: STM Tosilate.lcb**
Description	**: Analytical Method Validation-Sultamicillin Tosilate**

Chromatogram



1 Det.A Ch1 /217nm

PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.02	5338899	100.00	Sultamicillin
Total		5338899	100.00	

CHROMATOGRAM-39

Solution stability:-

Solution stability of Sultamicillin at bench top and in refrigerated condition over a period of few hours was studied by injecting test solutions at regular intervals of one hour as per test method. The solution prepared for system precision is used.

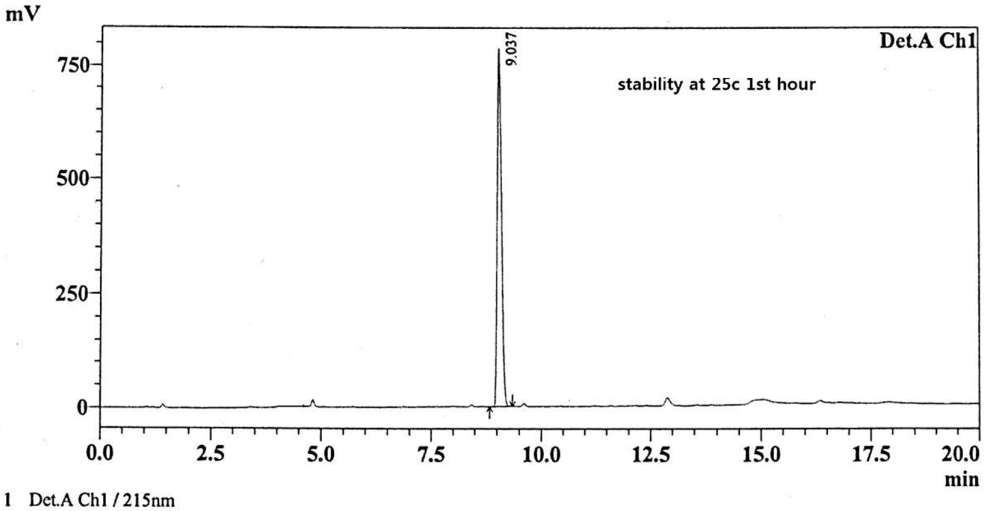
Acceptance Criteria

The solution is said to be stable if the % variability of the assay value does not exceed by1.5 with that of initial. Hence the Bench top solution should be used within 4 hours and refrigerated solution can be used within 11 hours.

Time in hours	Bench top stability (25ºC)			Refrigerated Condition (4-6 ºC)		
	Response	Area %	%variability	Response	Area %	% variability
Initial	5314076			5366674		
1	5311386	99.95	0.05	5363788	99.95	0.05
2	5306941	99.87	0.13	5355219	99.79	0.21
3	5257571	98.94	1.06	5350625	99.70	0.30
4	5232383	98.46	1.54*	5331925	99.35	0.65
5	5165822	97.21	2.79	5320804	99.15	0.85
6	5098639	95.95	4.05	5313440	99.00	0.99
7	5063835	95.29	4.71	5304785	98.85	1.15
8				5303020	98.81	1.18
9				5301082	98.78	1.22
10				5298173	98.72	1.27
11				5297131	98.70	1.30*
12				5276193	98.31	1.69

Table:-15

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Solution Stability at 25-deg 1st hour
Tray#	: 1
Vail#	: 14
Injection Volume	: 10 uL
Data Filename	: QCLC003 29101003.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

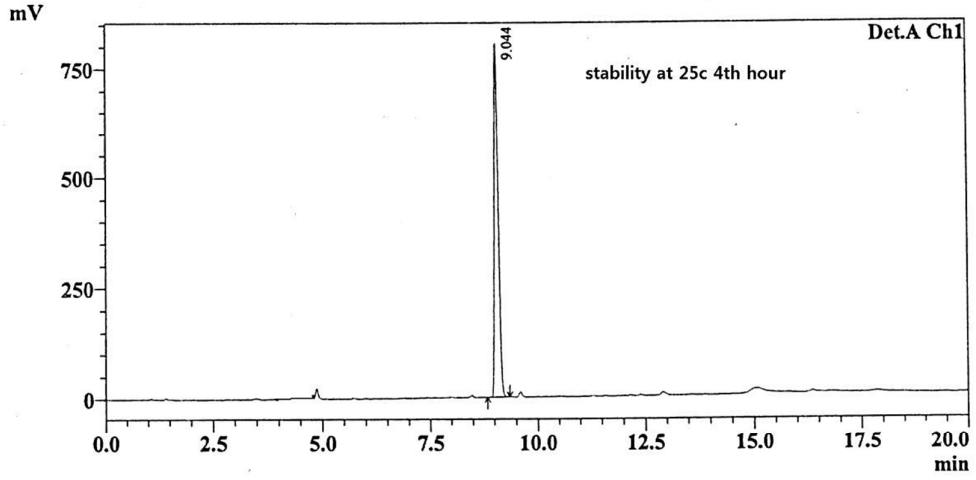
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.04	5311386	100.00	Sultamicillin
Total		5311386	100.00	

CHROMATOGRAM-40

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Solution Stability at 25-deg 4th hour
Tray#	: 1
Vail#	: 18
Injection Volume	: 10 uL
Data Filename	: QCLC003 29101007.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

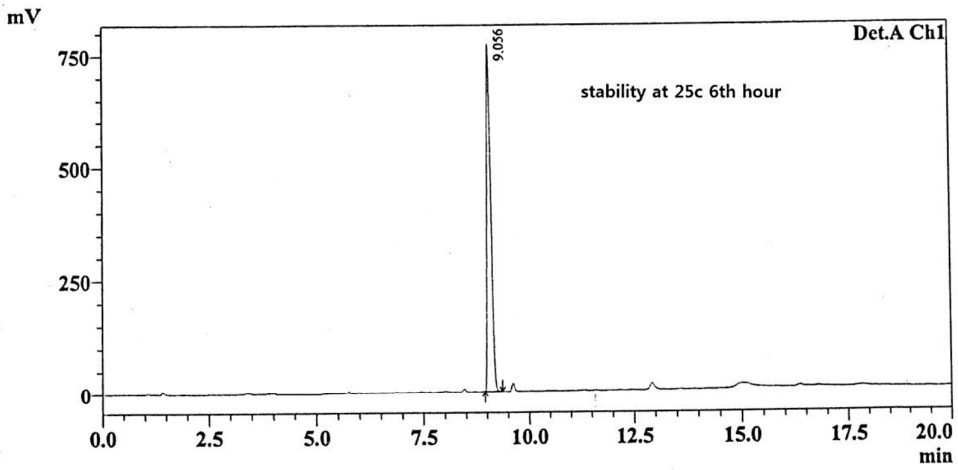
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.04	5232383	100.00	Sultamicillin
Total		5232383	100.00	

CHROMATOGRAM-41

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Solution Stability at 25-deg 6th hour
Tray#	: 1
Vail#	: 22
Injection Volume	: 10 uL
Data Filename	: QCLC003 29101011.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

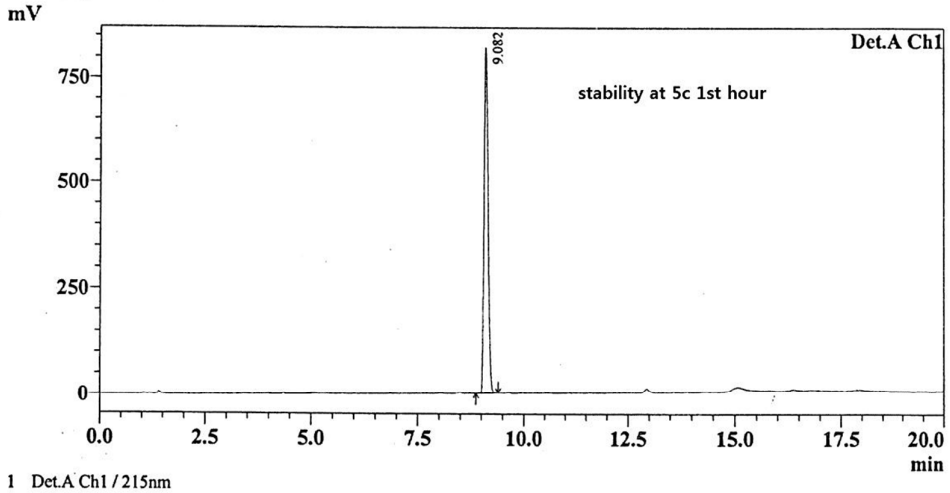
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.06	5098639	100.00	Sultamicillin
Total		5098639	100.00	

CHROMATOGRAM-42

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Solution Stability at 5-deg 1st hour
Tray#	: 1
Vail#	: 13
Injection Volume	: 10 uL
Data Filename	: QCLC003 29101002.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

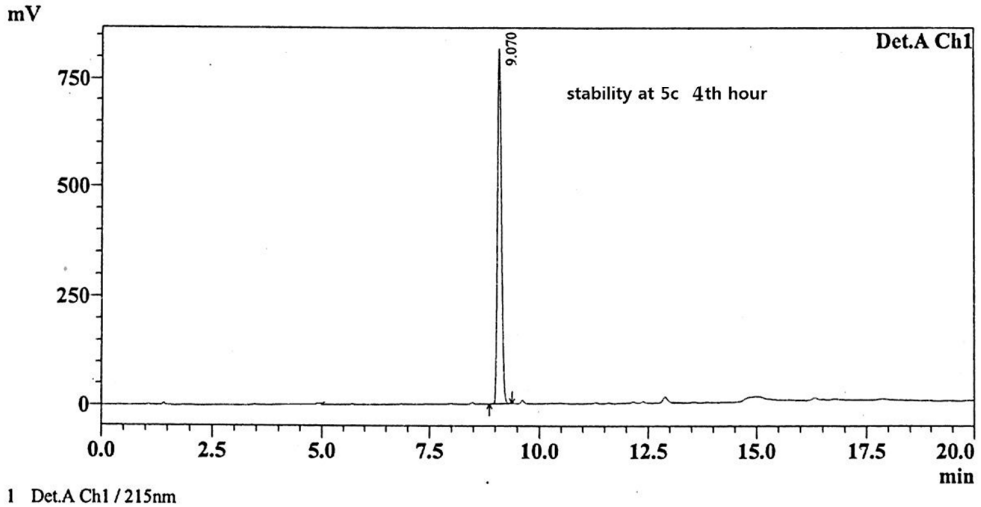
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.08	5363788	100.00	Sultamicillin
Total		5363788	100.00	

CHROMATOGRAM-43

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Solution Stability at 5-deg 4th hour
Tray#	: 1
Vail#	: 29
Injection Volume	: 10 uL
Data Filename	: QCLC003 29101018.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

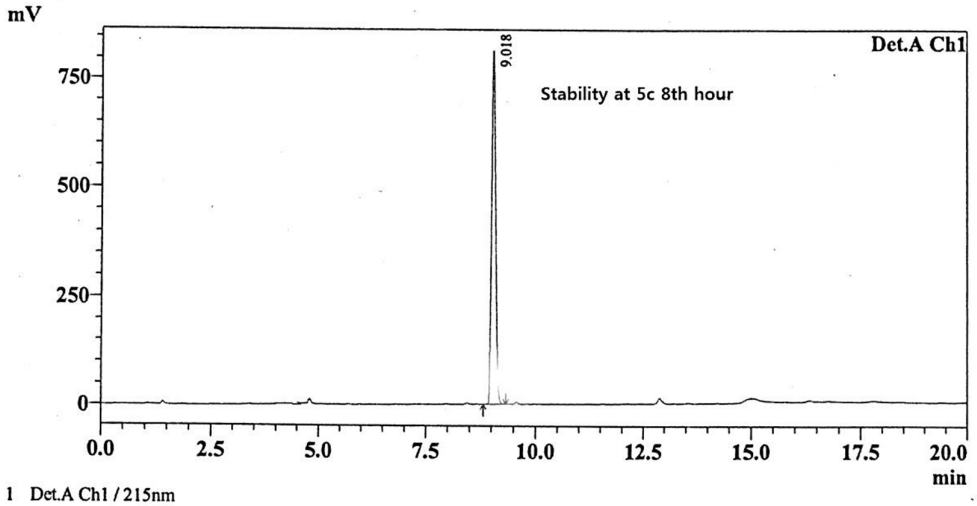
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.07	5331925	100.00	Sultamicillin
Total		5331925	100.00	

CHROMATOGRAM-44

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Solution Stability at 5-deg 8th hour
Tray#	: 1
Vail#	: 21
Injection Volume	: 10 uL
Data Filename	: QCLC003 29101010.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

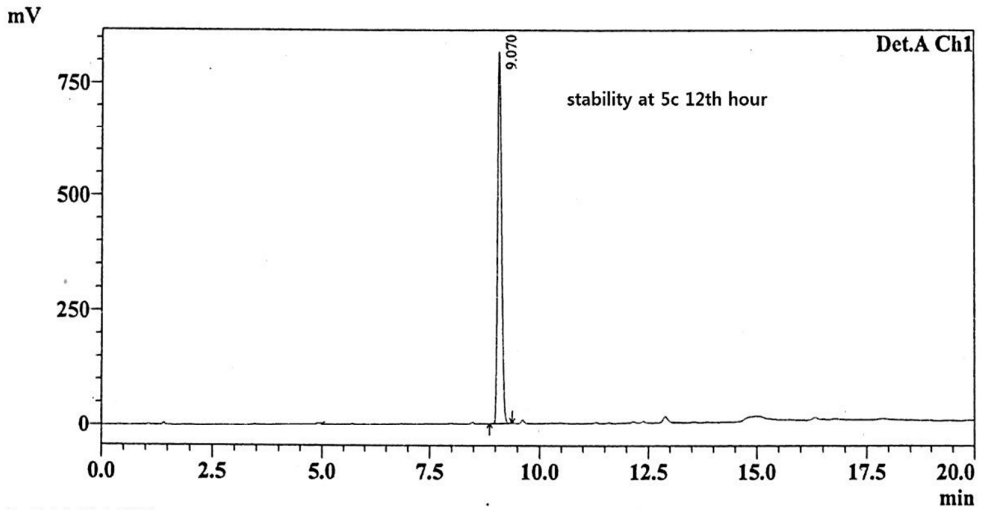
PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.02	5303020	100.00	Sultamicillin
Total		5303020	100.00	

CHROMATOGRAM-45

Instrument ID.No.	: QCLC-003
Sample Name	: Sultamicillin Tosilate
Sample ID	: Solution Stability at 5-deg 12th hour
Tray#	: 1
Vail#	: 29
Injection Volume	: 10 uL
Data Filename	: QCLC003 29101018.lcd
Method Filename	: Sulatamicillin Tosilate (EP).lcm
Batch Filename	: STM Tosilate.lcb
Description	: Analytical Method Validation-Sultamicillin Tosilate



1 Det.A Ch1 / 215nm

PeakTable

Detector A Ch1 215nm

Peak#	Ret. Time	Area	Area %	Name
1	9.07	5276193	100.00	Sultamicillin
Total		5276193	100.00	

CHROMATOGRAM-46

Results and discussion

RESULTS AND DISCUSSION

The working conditions for the HPLC method was established for sultamcillin tosilate and then applied on pharmaceutical dosage forms.

Various mobile phase systems were prepared and used. Phosphate buffer : Acetonitrile in the ratio of 20 : 80gave the better resolution and sensitivity.

The detection was carried out by using UV-visible detector at 215nm.Among the several flow rates tested (0.5-2.0), the flow rate of 1.0ml/min ml was the best for the drug with respect to location and resolution of analytical peaks.

The retention time is 9.05min for sultamcillin tosylate. The number of theoretical plates was found to be 5248.20 for sultamcillin tosylate which indicates the symmetrical nature of the peak. The retention time for the drug was found to be 0-9 minutes. This parameter represents the specificity of the method.

System suitability parameters such as tailing factor, capacity factor, resolution and number of theoretical plates were calculated and the results are tabulated in table-5,6 and supported by the chromatogram-3 to 7.

From the linearity studies, the specified concentration range was determined. It was observed that sultamcillin tosylate is linear in the range of 50-150 % for the target concentrations. The linearity range of 12.5-37.5µg/ml for sultamcillin tosylate found to obey the linearity with the correlation coefficient of 0.9996. The linearity curve for sultamcilllintosylate were shown in the figure-8. The results of analytical performance parameters were tabulated in table-8,9,10.

The validation of the proposed method was verified by system precision and method precision. The %RSD for system precision and method precision of sultamcillin tosylate was 0.17,0.8507 respectively.
The validation of the proposed method was verified by recovery studies. The percentage recovery range was found to be satisfied which is represented in the table-13.

The robustness studies were performed by changing wavelength and reports shown in the table-14.

The validation of the proposed method was also verified by solution stability which is represented-15

All the parameters including flow rate, temperature, detection wavelength and sensitivity were maintained constant throughout the procedure except for robustness studies.

Materials and Methods for Hptlc

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

Instrumentation

Name of sample applicator: Linomat IV with CAMAG 100µl syringe

Scanner name : CAMAG TLC scanner III

Software used : WINCATS

Chamber : CAMAG twin trough chamber (20X10)

Stationary phase : TLC silica gel 60 F254

Mobile phase : Acetonitrile: Methanol: Water (3.5:5:1.5 v/v/v)

Development time : 20mints

Development temperature : ambient

Detection : 215nm

Diluents : Methanol

Method development and optimization

Trial1 - methanol: acetone: ethyl acetate (5:2.5:2.5)

Trial2 – n-butanol: ethanol (1:1)

Trial 3- n-butanol: ethyl acetate: water (4:4:2)

Trial 4 – acetonitrile: ethyl acetate: methanol (4:4:2)

Trial 5 – ethyl acetate: methanol: water (2:0.5:7)

Trial 6- Acetonitrile: methanol: Water (3.5:5:1.5 v/v/v)

Out of the 6 trial made in the lab, the $6^{th}{}_{trial}$ was selected for further studies because when compare to other trials 6^{th} trial was found to be the best one .

Standard Solution

Weighed accurately 100 mg of sultamcillin tosilate into 100 ml standard flask, dissolved and diluted to volume with methanol.

Sample Preparation

Weighed and crushed 20 tablets, weigh blend equivalent to 100mg of sultamcillin tosilate into a 100 ml volumetric flask, sonicated for 30 minutes and made it up to the mark with methanol.

The percentage of drug content can be calculated using the given formula,

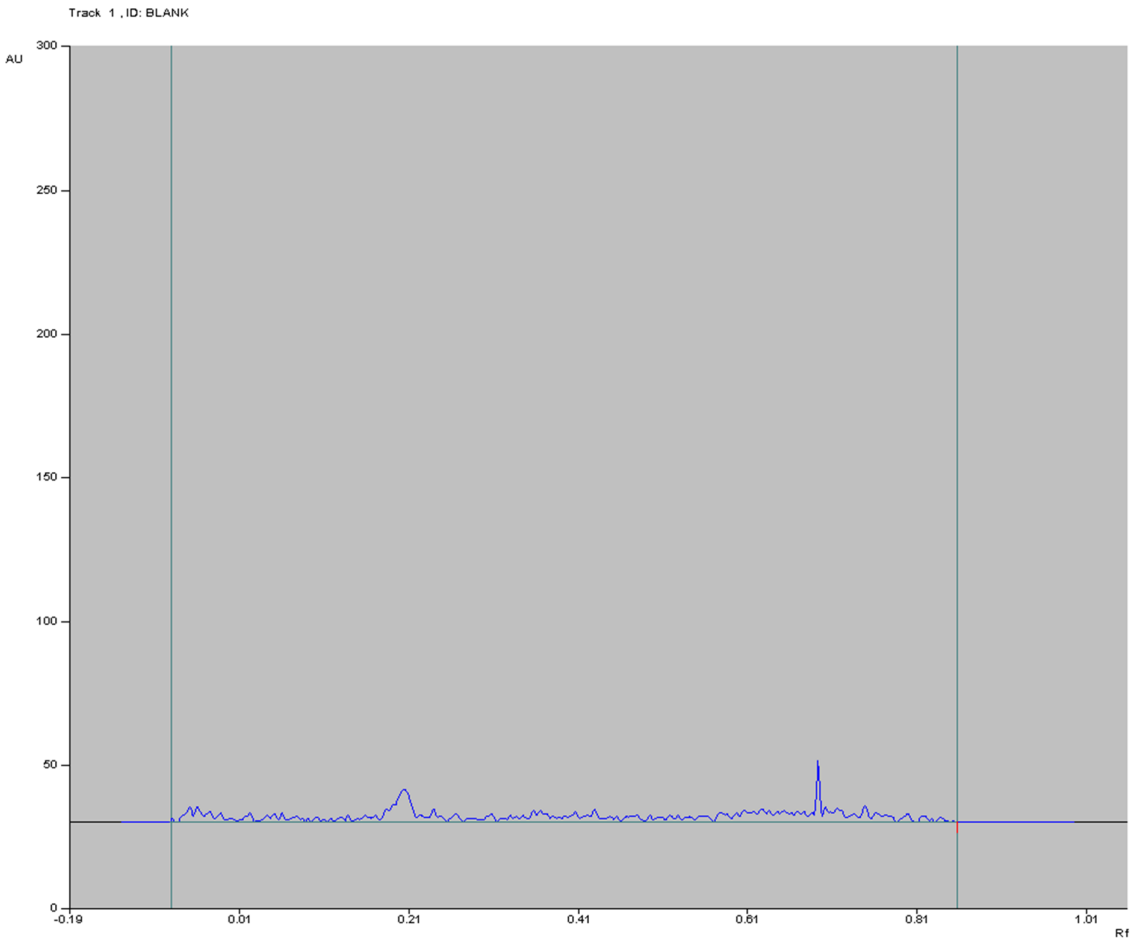
$$\% \text{ Drug content} = \frac{\text{Sample area} \times \text{STD D.F.} \times \text{Avg. wt.} \times 100}{\text{Std. area} \quad \text{SPL D.F} \quad \text{LC}}$$

Where,

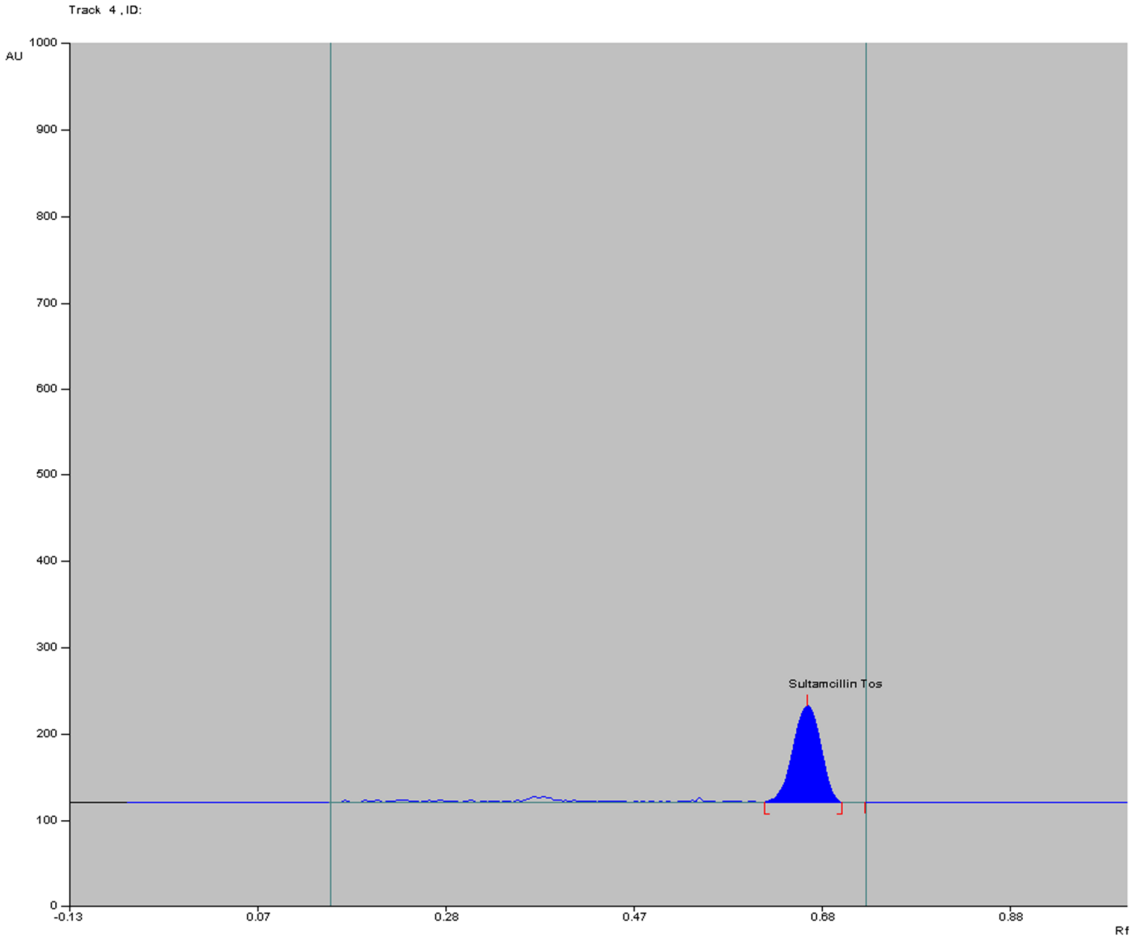
STD D.F = Standard dilution factor

SPL D.F = Sample dilution factor

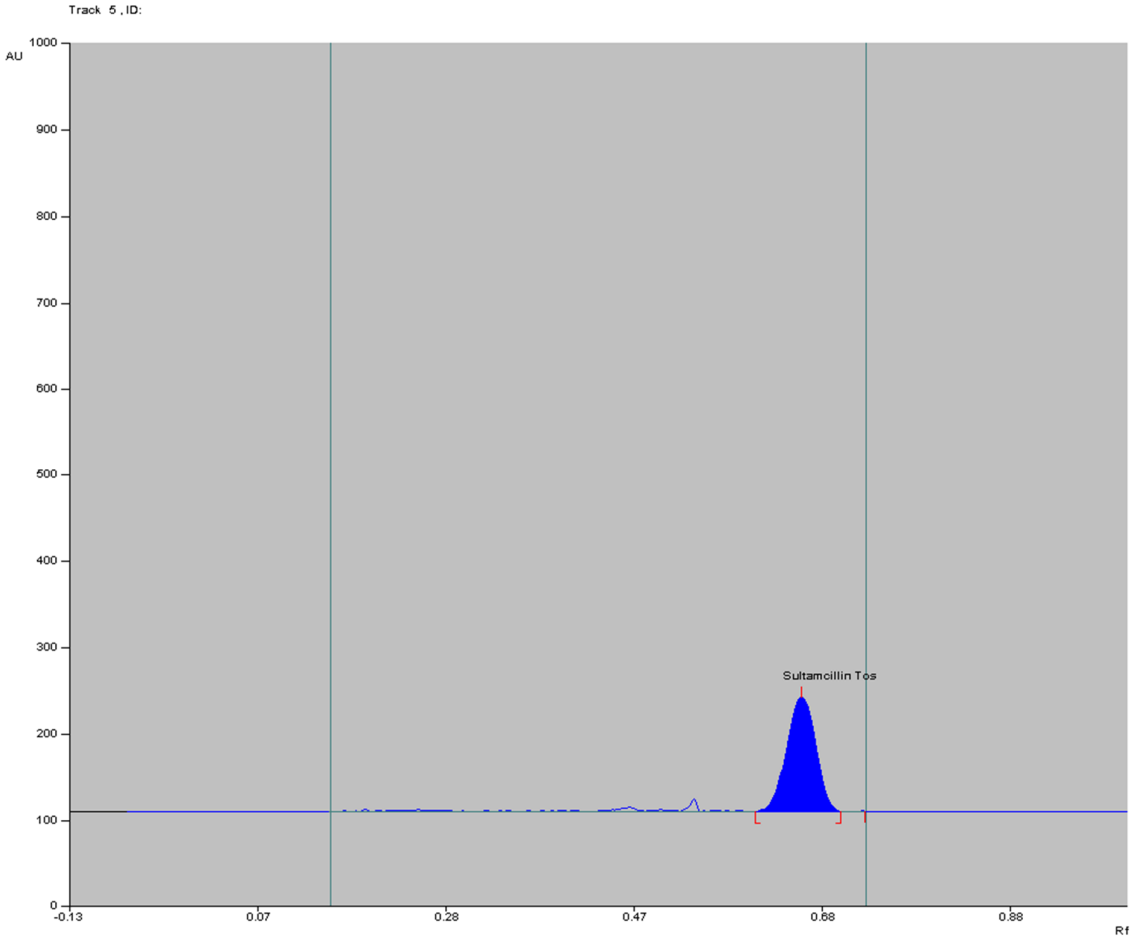
L C = Label claim



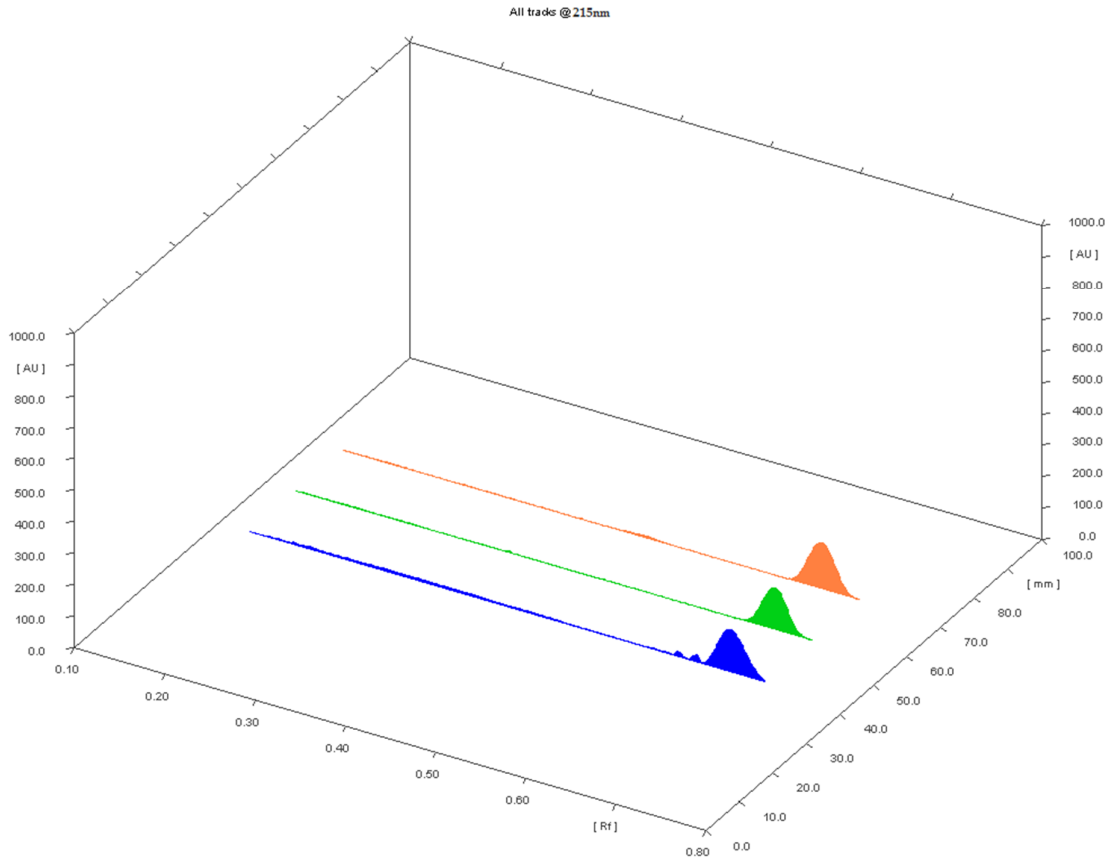
Chromatogram-47(blank)



Chromatogram-48(standard)



Chromatogram-49(sample)



HPTLC densitogram For Assay of sultamcillin tosilate

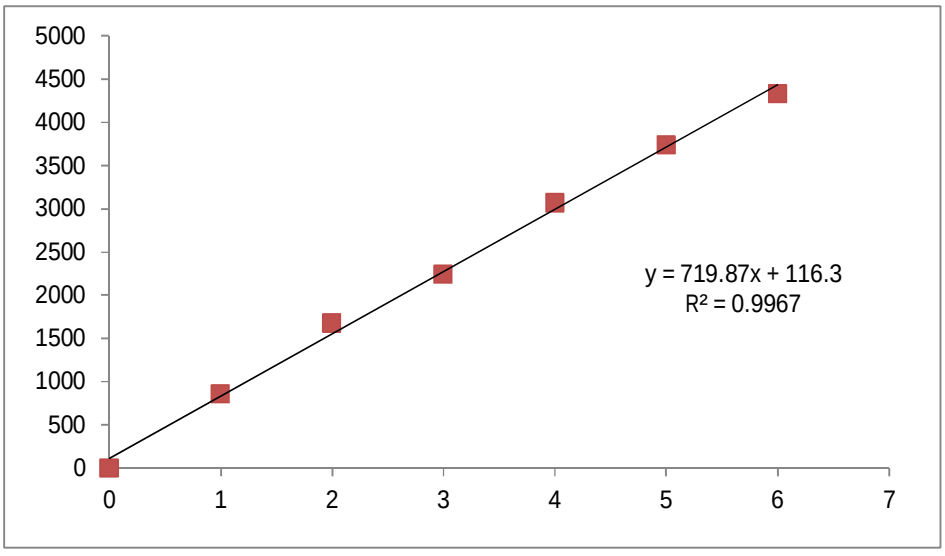
Table 16 Assay of sultamcillin tosilate

Sample concentration (µg/spot)	Peak area	Standard concentration (µg/spot)	Peak area	Amount (µg/spot)	% purity
3	2329.9	3	2338.9	39.8563978	99.61754393
3	2328.6	3	2338.9	39.70350789	99.56196094
3	2331.9	3	2338.9	39.85282561	99.70305622

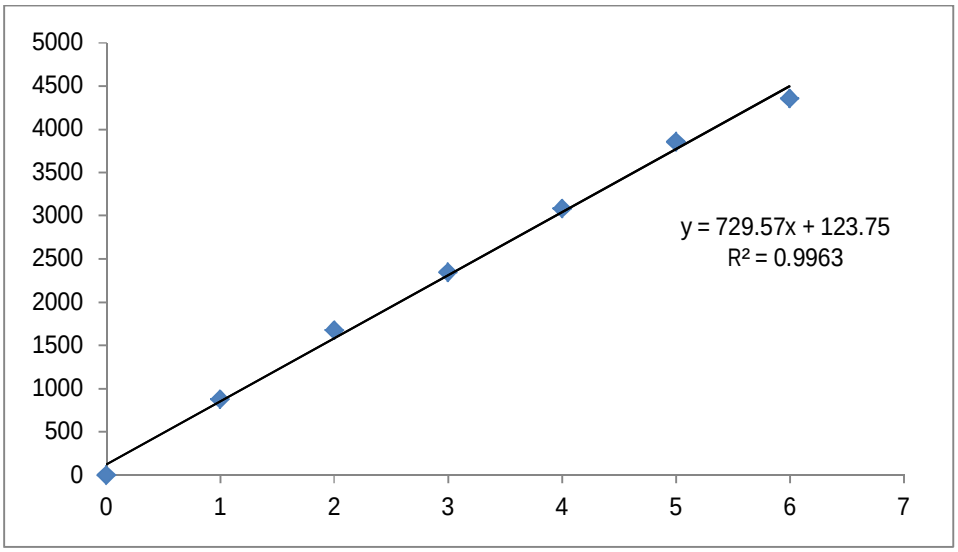
Conclusion The estimated percentage purity was found to be close to 100% which prove the accuracy of the method.

LINEARITY

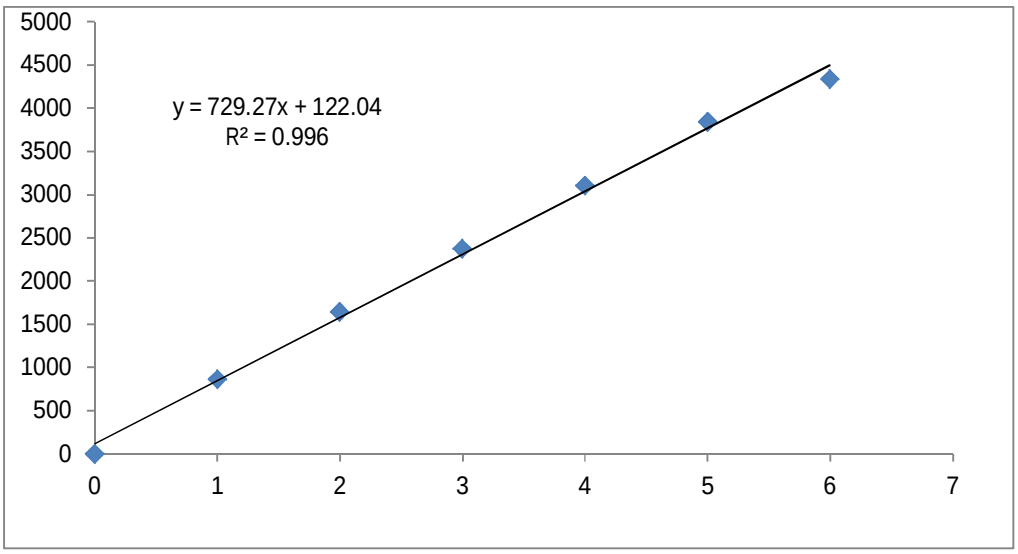
The response for the drug was linear (R^2= 0.9963) in the concentration range between 1-6 mcg/spot. The mean (±RSD) values of slope, intercept and correlation coefficient were 726.25 (±1.53) and 120.71 (±0.856) and 0.9963 (±0.236), respectively.



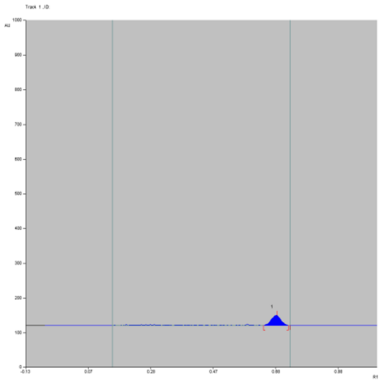
HPTLC for sultamcillintosilate LINEARITY-1**(FIG-9)**



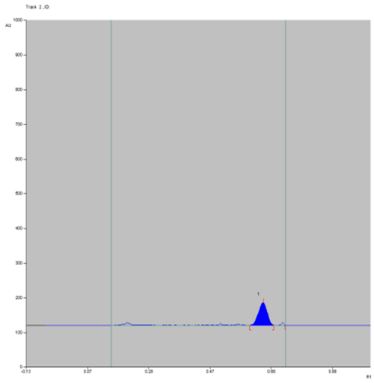
HPTLC for sultamcillin tosilate LINEARITY-**2 (FIG-10)**



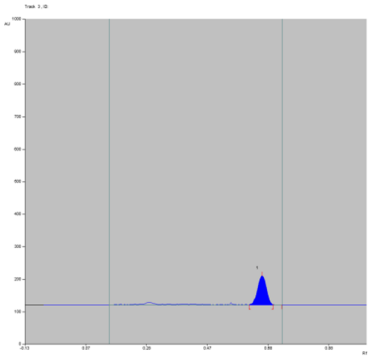
HPTLC for sultamcillin tosilate LINEARITY-3**(FIG-11)**



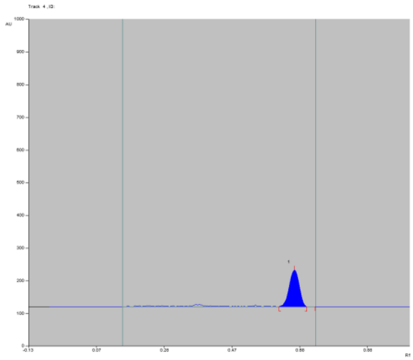
Chromatogram-50



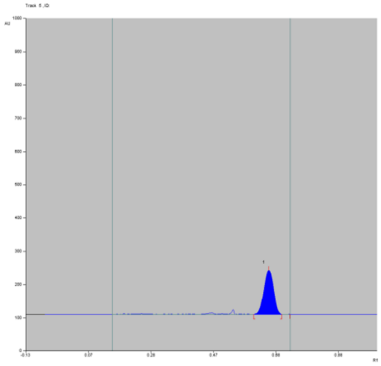
Chromatogram-51



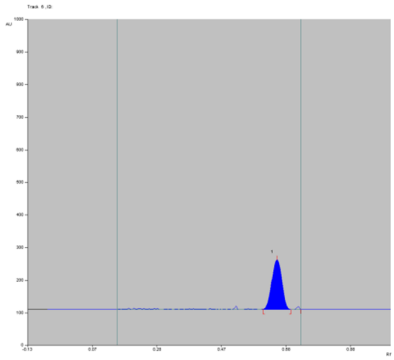
Chromatogram-52



Chromatogram-53

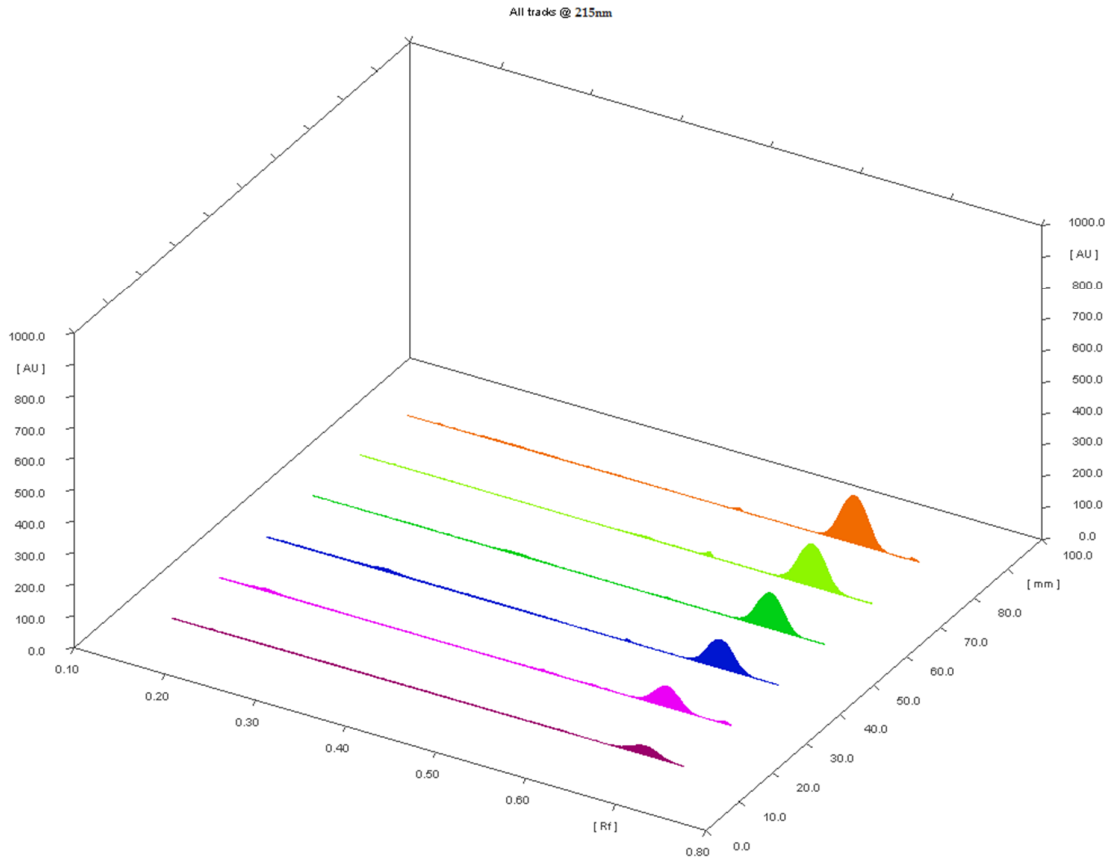


Chromatogram-54



Chromatogram-55

Linearity chromatograms



HPTLC densitogram- for linearity

Linearity data of the method

Table 17

Concentration(µg/spot)	**Peak area**	**Peak area**	**Peak area**	**R_f value**
0	0	0	0	0
1	859	875	865.2	0.68
2	1608.6	16745.5	1645.2	0.65
3	2243.2	2345.2	2375.5	0.66
4	3075.7	3085.2	3105.5	0.65
5	3741.4	3854.7	3842.5	0.65
6	4332.5	4352.6	4334.9	0.65

$y = mx + c$

where,

y= correlation coefficient

m= slope

c=intercept

Linearity parameters of sultamcillin tosilate

Table 18

Linearity	**Correlation coefficient(y)**	**Slope(m)**	**Intercept(c)**
Linearity 1	0.9967	719.91	116.34
Linearity 2	0.9963	729.57	123.75
Linearity 3	0.9960	729.27	122.04
Average	0.9963	726.25	120.71
Std. deviation			3.87791

Acceptance criteria: The Linear Regression coefficient should not be less than 0.97

Conclusion: The linearity study reveals that the concentration is linear in the range of 1-6 mcg/spot. The linear coefficient is 0.9963. Hence complies.

LIMIT OF DETECTION

LOD=3.3× Std. Deviation/Average Slope

=0.0177µg/spot

LIMIT OF QUANTITATION

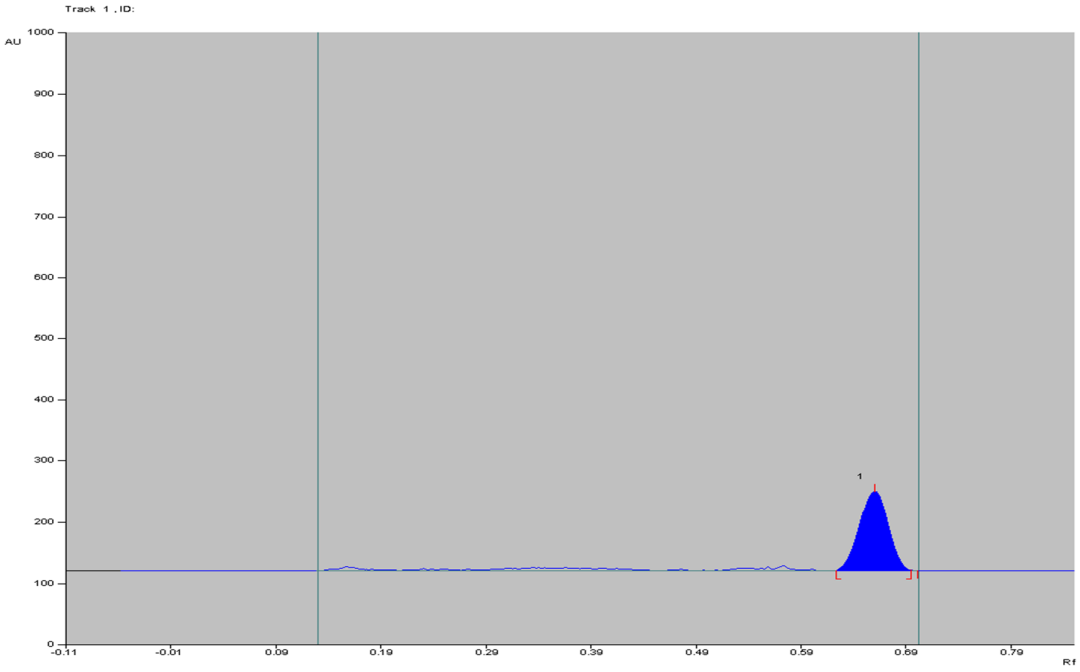
LOQ=10× Std. Deviation/Average Slope

=0.05368µg/spot

PRECISION

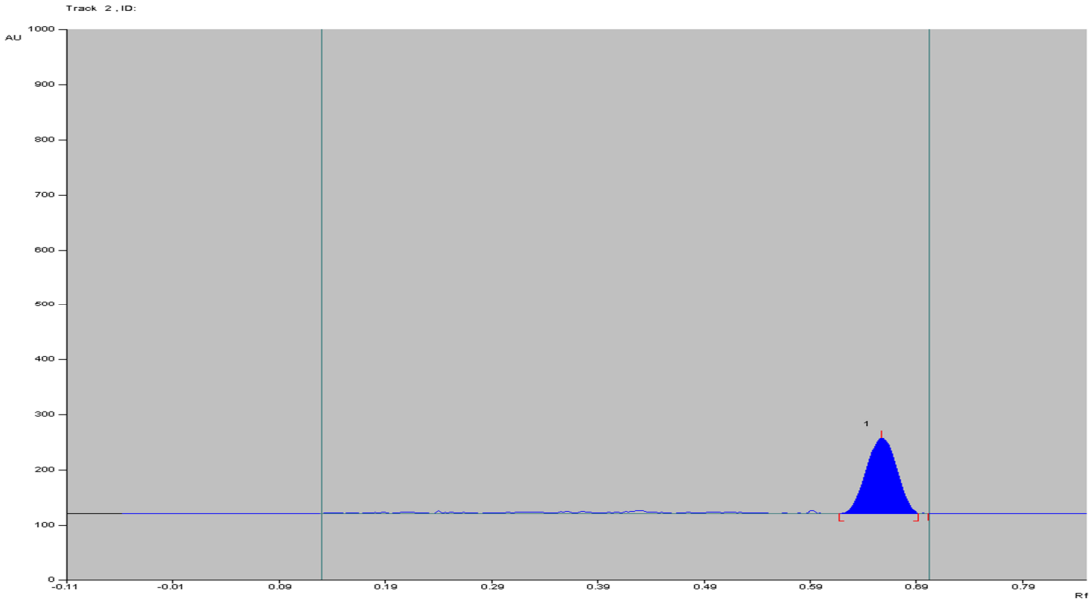
Table-19(Precision for Sultamcillintosilate)

concentration	Inter assay	Intra assay	
	Day 1	Day 1	Day 2
	Precision 1	Precision 2	Precision 3
3µg/Spot	2338.9	2334.5	2368.2
3µg/Spot	2334.2	2341.2	2334.2
3µg/Spot	2312.5	2354.1	2345.9
3µg/Spot	2335.2	2337.5	2387.2
3µg/Spot	2342	2354.6	2341.2
3µg/Spot	2335.4	2324.2	2355.9
Mean	2333.0333	2341.0166	2355.4333
SD	10.46989	11.7758	19.57883
%RSD	0.4487	0.5030	0.8399



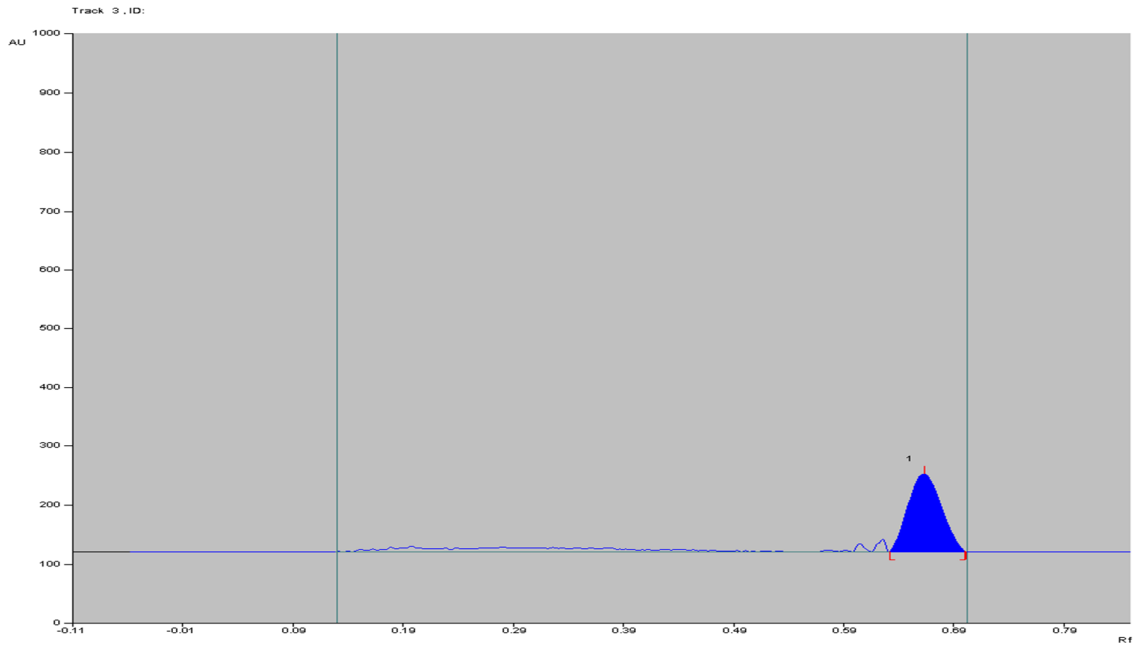
Precision-1

Chromatogram-56



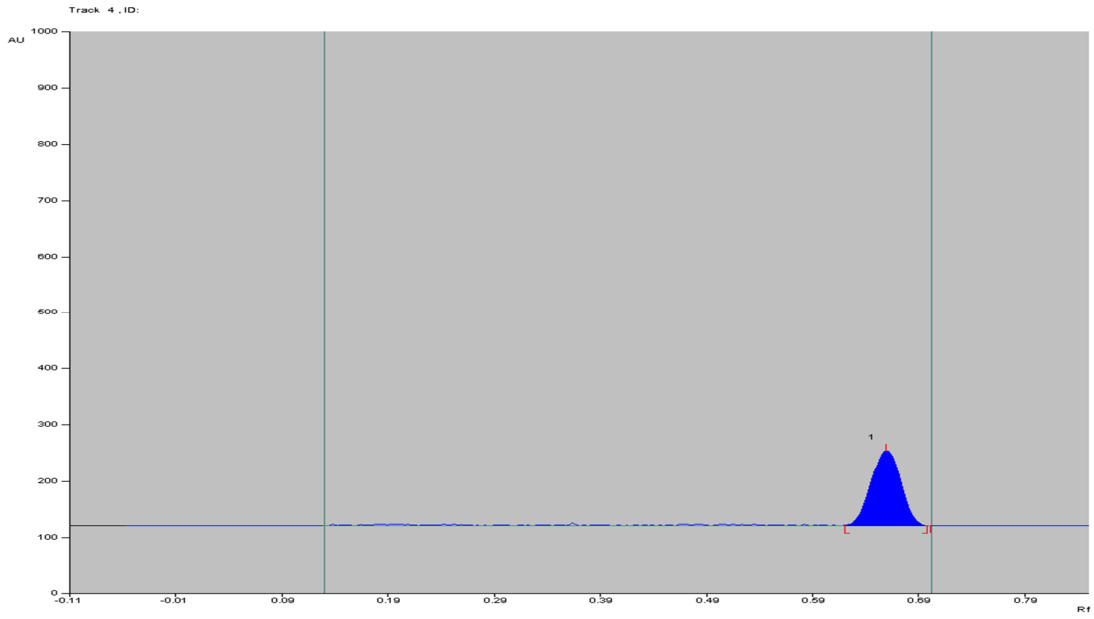
Precision-2

Chromatogram-57



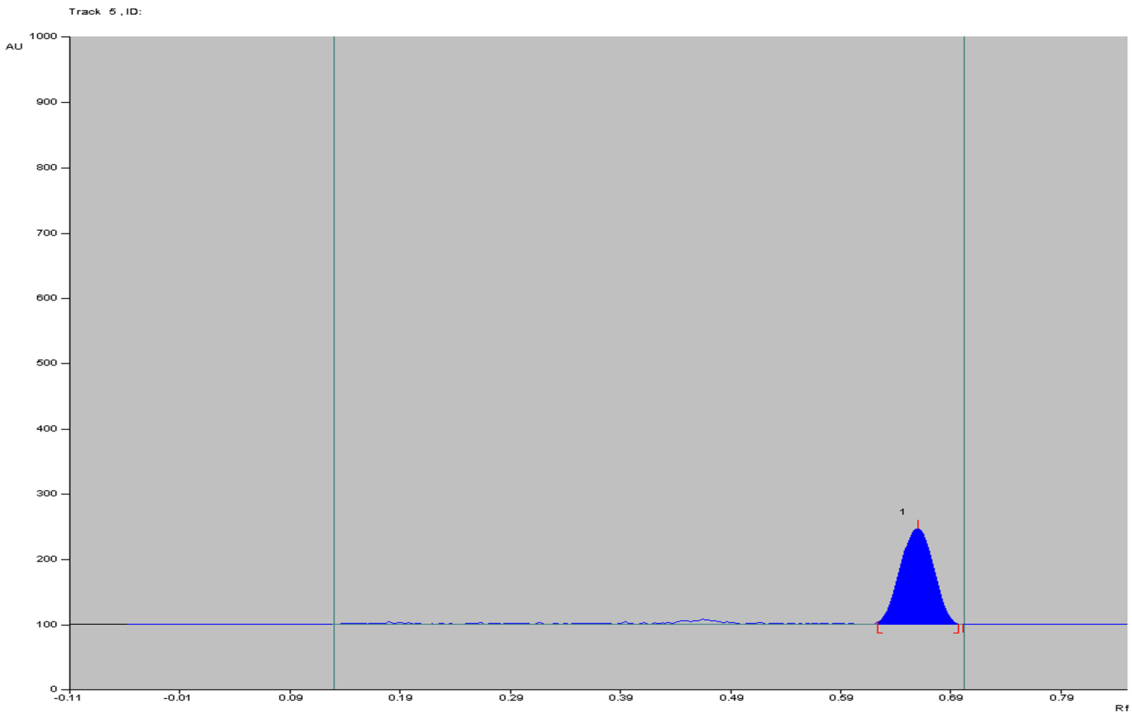
Precision-3

Chromatogram-58



Precision-4

Chromatogram-5



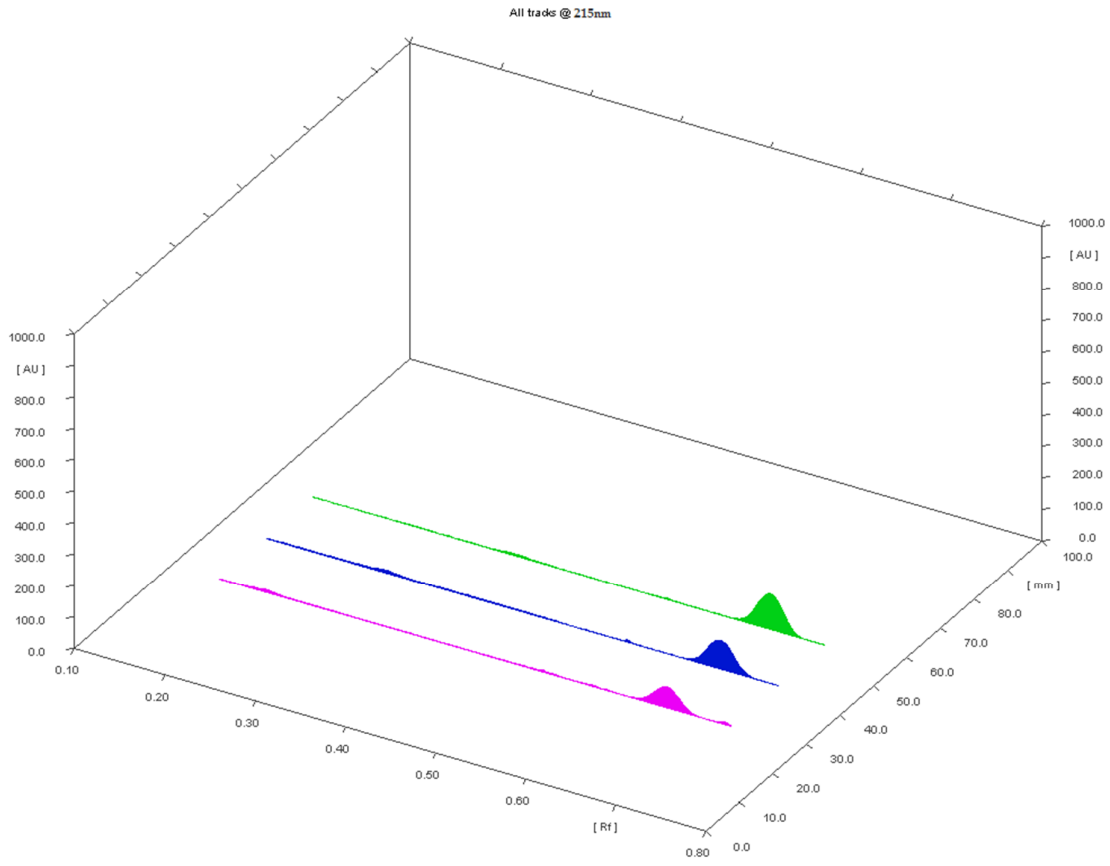
Precision-5

Chromatogram-60

Acceptance criterion: The % RSD calculated must not be more than 2.00.

Conclusion: The Standard deviation, Relative Standard deviation and Standard error were found to be low for the drug and hence prove that the method is precise.

Accuracy:-

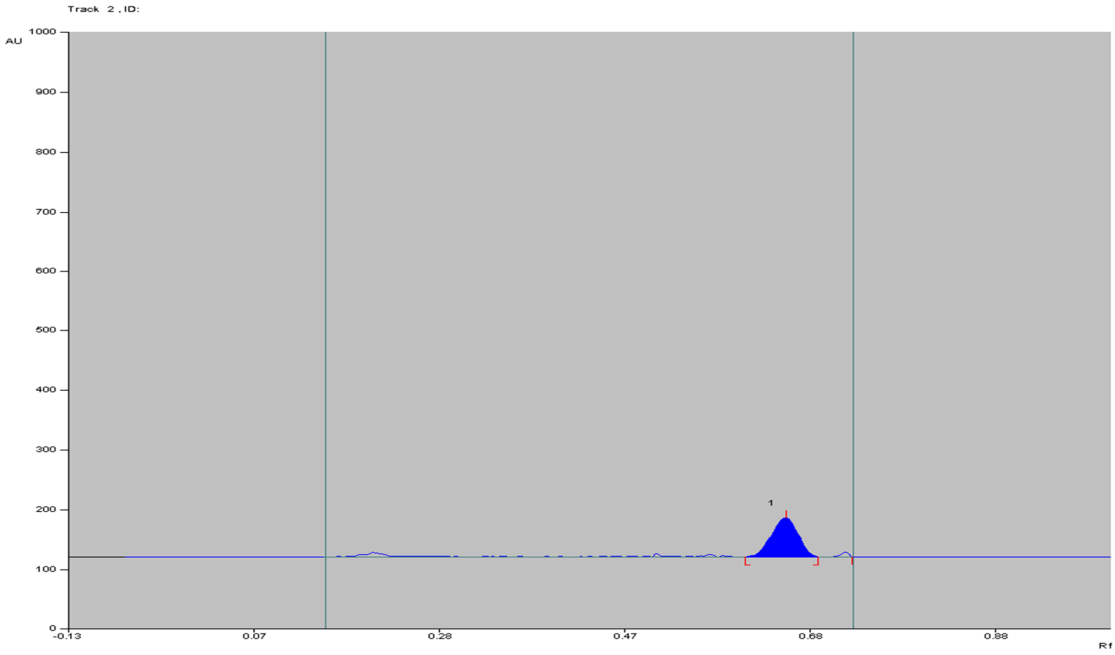


HPTLC densitogram for accuracy

Table-20 (Accuracy for sultamcillin tosilate)

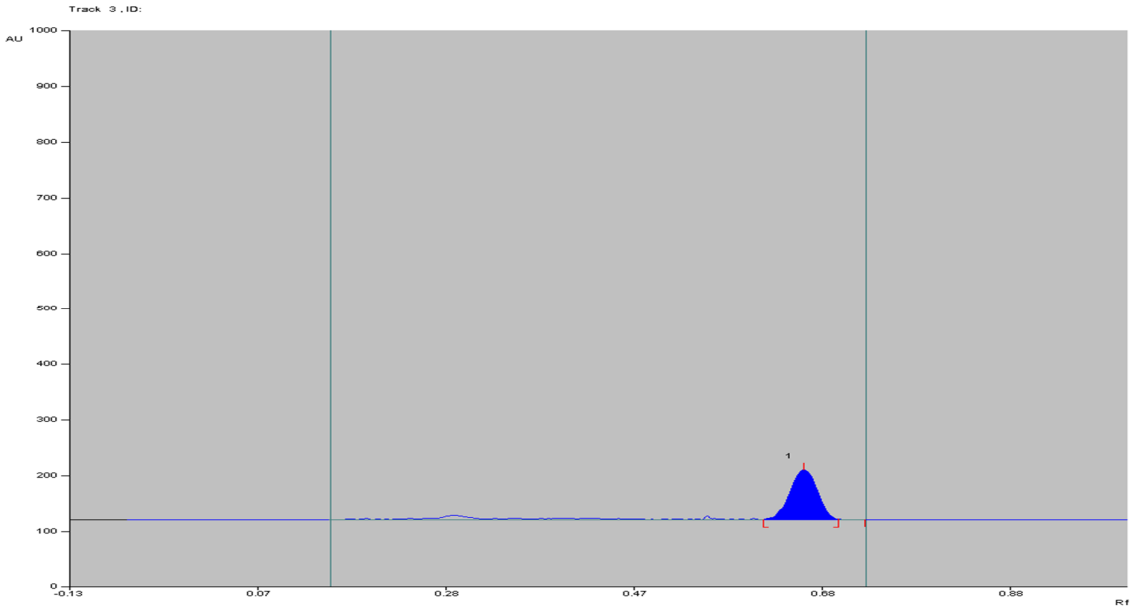
Sample concentration(µg/spot)	Peak area	Standard concentration (µg/spot)	Peak area	Amount found	Percentage found	Average
2	1680.6	2	3901	100.77519	100.775	100.0853
2	1675.2	2	3986.3	99.7942	99.794	
2	1672.4	2	3975.1	99.68705	99.687	
3	2324.5	3	5549.1	100.13552	100.135	99.832
3	2345.6	3	5534.9	99.67407	99.674	
3	2341.9	3	5498.8	99.26042	99.260	
4	3105.6	4	7286.6	100.30632	100.306	100.0773
4	3101.1	4	7445	100.28475	100.284	
4	3098.5	4	7478.7	99.64272	99.642	

Conclusion: The estimated percentage purity was found to be close to 100% which prove the accuracy of the method.



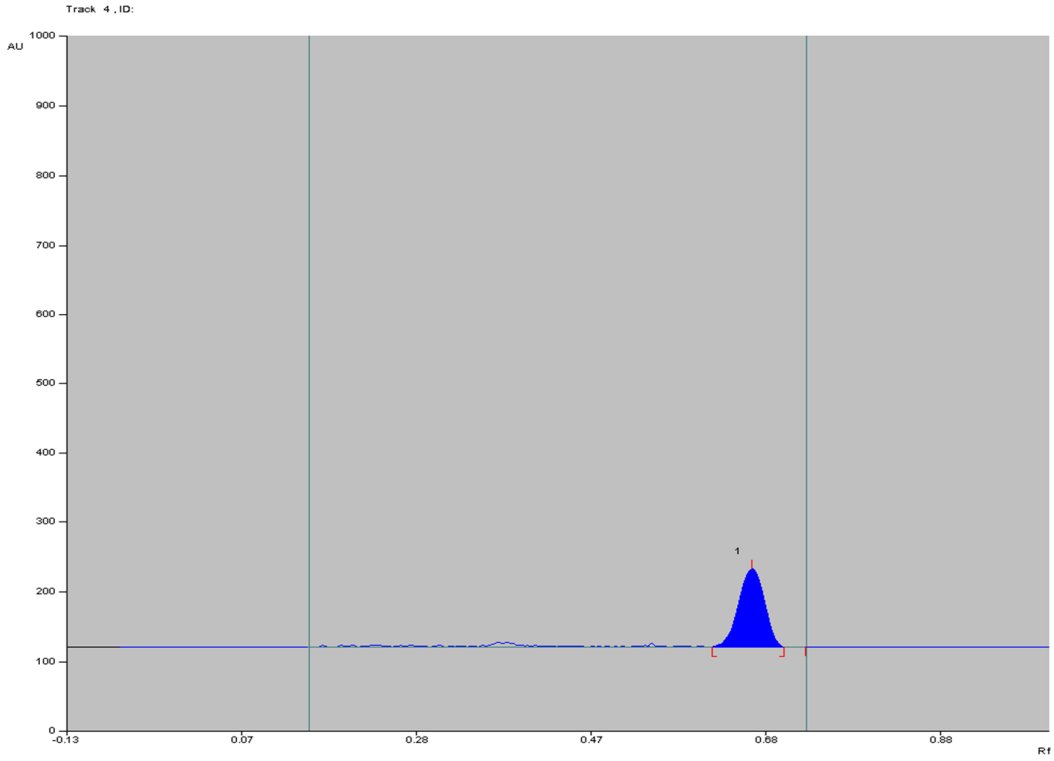
Accuracy-1

Chromatogram-61



Accuracy-2

Chromatogram-62



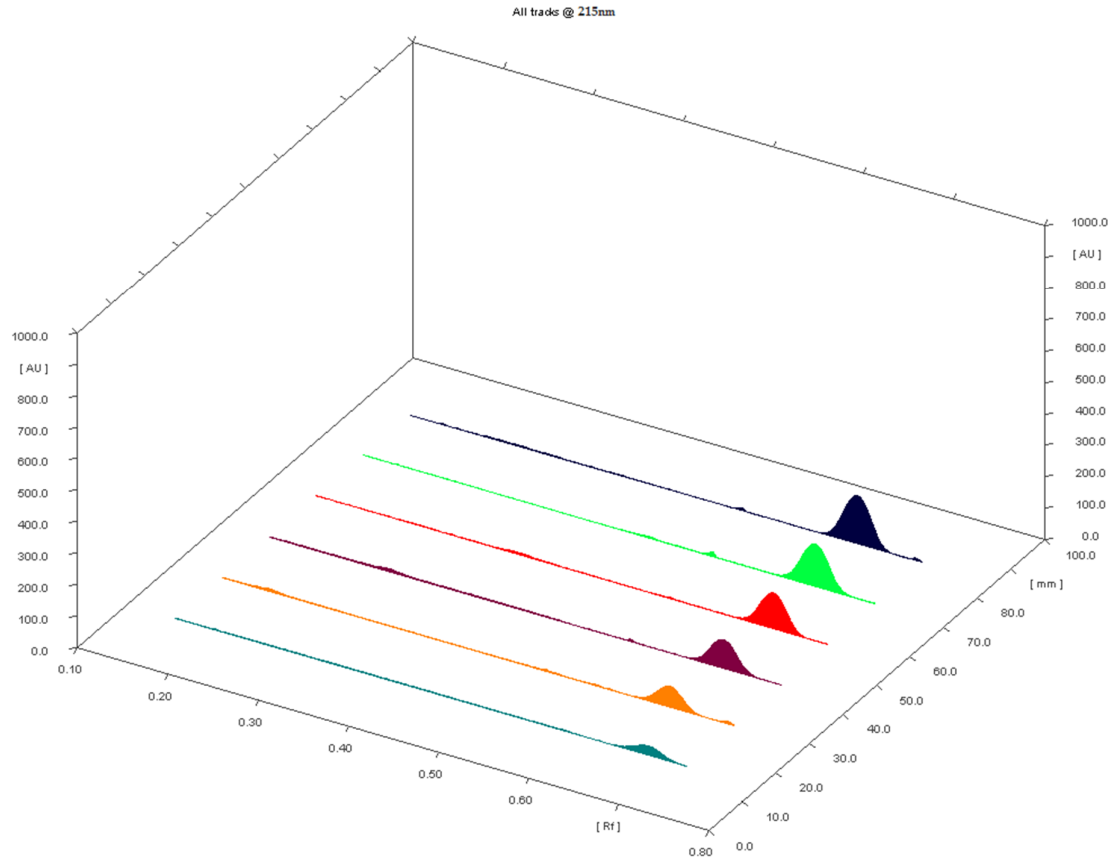
Accuracy-3

Chromatogram-63

RECOVERY

Table-21(Recovery for sultamcillin tosilate)

S.No	Amount present (mcg/spot)	Amount added (mcg/spot)	Area	Amount found (mcg/spot)	% recovered
1	1	0	857	1.022706385	102.27064
2	1	1	1674.6	2.012542574	101.25426
3	1	2	2354.9	2.997188733	99.718873
4	1	3	3112.5	4.009406931	100.94069
5	1	4	3831.4	5.004919717	100.49197
				Mean	100.9346
				Std.dev	0.9436



HPTLC densitogram for recovery of sultamcillintosilate

Conclusion: The estimated percentage recovery was found to be close to 100% and hence prove the recovery of the method. The percentage recovery of sultamcillintosilate in the marketed formulation tablets was found to be in the range of 99.7188%-102.270% and which is well within the acceptance limit of 97% - 103%w/w.

Results and discussion

RESULTS AND DISCUSSION

The working conditions for the HPTLC method was established for sultamcillin tosylate and then applied on pharmaceutical dosage forms.

Various types of solvent systems were evaluated to obtain an optimum resolution of drug. Acetonitrile: Methanol: Water (3.5:5:1.5 v/v/v) gave the better resolution and precision. The detection was carried out at 215nm

The retention factor is 0.65 for sultamcillin tosylate. The quantitative estimation report sultamcillin tosylate given in table-21.

From the linearity studies, the specified concentration range was determined. The linearity range is 1-6 µg/spot for sultamcillin tosylate found to obey the linearity with the correlation coefficient of 0.9963. The linearity curve for sultamcillin tosylate was shown in the figure-15,16,17.

The validation of the proposed method was verified by system precision. The %RSD for system precision for sultamcillin tosylate was calculated.

The validation of the proposed method was also verified by recovery studies. The percentage recovery range was found to be satisfied which is represented in the table-26.

The parameters including flow rate, temperature, detection wavelength and sensitivity were maintained constant throughout the procedure.

VALIDATION PARAMETERS

Table-22

Parameters	**Results**
Limit of detection	0.0177µg/spot
Limit of quantification	0.05368µg/spot
Linearity range(µg/spot)	1-6µg/spot
Correlation coefficient	0.9963
Slope	726.25
Intercept	120.71
Percentage Recovery	99.7188%-102.270%
Accuracy	99.975%
Precision(% RSD)	0.5972
Assay(% purity)	99.5619%-99.7030%

Summary and conclusion

SUMMMARY AND CONCLUSION

SUMMARY OF VALIDATION FOR SULTAMCILLIN TOSILATE BY HPLC

	Parameter	Observation	Acceptance criterion	Result
1	**System suitability**	0.54 1.10 5248.2	RSD NMT 2.0% Tailing NMT 2.5 Theoretical plates NLT 1500	Complies
2	**Specifity** i) Retention Time ii) By Placebo method	No interference at principle peak RT	There should be no interfering peak from the placebo and diluent	Complies
3	**Method precision**	0.8507	RSD NMT 3.0 %	Complies
4	**Linearity**	0.9996	NLT 0.99	Complies
6	**Accuracy**	99.78 – 100.80	98.0% to 102.0%	Complies
7	**Robustness** AT 213 AT 217	0.7933 0.7927	RSD NMT 5.0 %	Complies
8	**Solution stability** solution stability-table top At 1st hour At 4th hour	0.05 1.54	± 1.5%	Complies
	solution stability-refrigerated At 1st hour At 12th hour	0.05 1.69	± 1.5%	Complies

SUMMARY OF VALIDATION FOR SULTAMCILLIN TOSILATE BY HPTLC

Parameters	**Results**	**Acceptance criteria**
Limit of detection	0.0177µg/spot	---
Limit of quantification	0.05368µg/spot	---
Linearity range(µg/spot)	1-6 µg/spot	---
Correlation coefficient	0.9963	>0.9960
Slope	726.25	---
Intercept	120.71	---
Percentage Recovery	99.7188%-102.270%	97-103%w/w
Accuracy	99.975%	Should be close to 100%
Precision(% RSD)	0.5972	< 2%
Assay(% purity)	99.5619%-99.7030%	97 – 103%w/w

CONCLUSION

A **RP-HPLC** method was developed for the estimation of Sultamcillintosilate in a tablet dosage form utilizing HPLC SHIMADZU 2010 PROMINENCE -separation module withUV-VIS detector kromasilC18 3.5µ(100mm×4.6mm) Injection volume of 10 µl is injected and eluted with the mobile phase of buffer and Acetonitrile in the ratio of 80:20 which is pumped at the flow rate of 1.0ml/min and detected at 215 nm wavelength using uv-vis detector. The run time per sample is 20 min. The peaks for Sultamcillin tosilate were found at 9.05. The excipients in the formulation did not interfere in the accurate estimation of Sultamcillin tosilate. The developed method was validated for accuracy, precision, linearity, specificity, and sensitivity in accordance with International Conference on Harmonization guidelines .The proposed method is applied for determination for Sultamcillin tosilate new formulation. Since none of the methods is reported estimation of Sultamcillin tosilate in oral dosage form, this developed method can be used for routine analysis of component in formulation.

A simple, and selective **high performance thin layer chromatographic** method was developed and validated for estimation of Sultamcillin tosilate pharmaceutical dosage forms. The method employed precoated TLC aluminum plates with silica gel 60F254 as the stationary phase. The solvent system comprised Acetonitrile: Methanol:Water(3.5:5:1.5 v/v/v).

The retention factor is 0.65min for sultamcillin tosilate. Spectrodensitometric scanning integration was performed 215 nm. The polynomial regression data for the calibration graph showed good linear relationship with R^2 = 0.9963 in the concentration range of 1-6 µg/spot. The developed method was validated for precision and recovery. The proposed method is applied for determination for sultamcillintosilate.

Bibliography

Bibliography

1. Sharma BK. **Instrumentalmethods of chemical analysis.** 22nd Edn. Meerut; Goel Publishing House,Pp 1-4,289-296.

2. Connors KA. **A Textbook of Pharmaceutical Analysis**. 3rdEdn. Delhi, Wiley intersciencesInc; 1994, Pp 1-3.

3. **Principles of instrumental analysis** by Douglas .A. skoog, Donald M.West, 2nd Edn, P 1,839,856.

4. **Vogel's Text book of Quantitative Chemical Analysis**by Denney R.C., Bassett J.,Mendham J., 5th Edn, Pearson education 2003, P 11,229.

5. Gurdeep.R. Chatwal, Sham. K.Anand ,**Instrumental method of chemical analysis** , published by himalaya publishing house, fifth revised Edn P 2.568, 2.673, 2.617, 2.647, 2.624, 2.588, 2.656, 2.630.

6.Kasture A V, Wododkar S G, Mohadik K R, Morl H N, **Instrumental Methods of Pharmaceutical Analysis,** Volume-2, 19th Edn, NiraliPrakashan, New Delhi, 2010, Pp 6-7

7. Williard HH,Merritt LL, Dean JJA,FrankAS,**Instrumental methods of analysis,**CBS publishers and distributors,New delhi:1986,P 7 , Pp68-75

8.Swarbrick James and Boylan James. C. Encyclopaedia of pharmaceutical technology. New York: Marcel Dekker Inc; 1998,Vol 1, Pp 217-224.

9. Lindsay S. High Performance Liquid Chromatography.1st Edn. London: John Wiley and sons; 1991, Pp 30-45.

10. Kenneth A Connors. Pharmaceutical analysis.3rdEdn. Singapore: John Wiley Publishers; 1982, Pp 529-530.

11. Lough WJ, Wainer IWW, HPLC fundamental principles and practices, Blackie Academic and professional; 1991, Pp 52-67.

12.SatinderAhuja and Michael W.Dong.Handbook of Pharmaceutical Analysis for HPLC,Elsevier,2006Edn,P 6.

13.P.C.Sadek,P.W.Carr,Journal of chromatographic science,1983, P 291

14. Joel K. Swadesh ,**HPLC-Practical and Industrial Applications** edited by, Charles H. Lochmuller, Series Editor Duke University Analytical ChemistrySeries, Second Edn P 11,12, 152-156.

15 Hobart H Willard, Lynne L Merritt, John A Deen, Frank A Settle, **Instrumental method of analysis** , published by CBS publishers, P 600-603.

16 Lloyd R. Snyder, Joseph.J. Kirkland, Joseph.L.Glaich,**Practical HPLC method development** ,2nd Edn, P 9,668

17. Sherma J, Fried B. **Handbook of thin layer chromatography**. 3rd Edn. New York: Marcel Dekker Inc; 2003,P 3-4.

18. Gennaro A.R., Remington.,**The sciences and practice of pharmacy**, 28th Edn, Luppincott, Williams and Wilkins, Baltimore, Maryland, USA, 2000, P 534 –549.

19. Jeffery G.H., Denney R.C., Bassett J., Mendham J., **Vogel's Text book of Quantitative Chemical Analysis**, 5th Edn, Pearson education 2003, P 216.

20. Beckett A.H., and Stenlake J.B., **Practical Pharmaceutical Chemistry**, 4thEdn, CBS publishers and distributors, 1997, P 275.

21 .Sethi PD**. Quantitative analysis of drugs in pharmaceutical formulations**.3rd Edn. NewDelhi: CBS Publisher; 1997,Pp 51-64,17-19

22. Peter EW. **Thin layer chromatography: A modern practical approach**. UK: The royal society of chemistry; 2005, Pp 6-154.

23 . Quality Assurance Guide**,** Organization of pharmaceutical producers of India, section 7.1 Pp 1-20.

24. Sop validation studies Indian Pharma guidance Academy Nagpur 1996 ,Pp 1-3).

25. Sharma P.P., **How to practice GLP Good laboratory practice,**Vandana Publications, 2000, Pp 148–156.

26. United States of pharmacopoeia, 26th Edn US Pharmacopoeia convention, Rock Ville M.D. 2003 P 1151

27. Validation of Analytical procedures / Methodology, ICH harmonized tripartite guidelines, 1996, Pp 1–8.

28. The United States pharmacopoeia-24, United States Pharmacopieal convention Inc., Rock Ville, 2000, Pp 1262-1263.

29. Gupta S.P., Statistical Methods, 10th Edn, Chand and Co Publishers, New Delhi,1999, Pp9-13.

30. Friedel, Heather A.; Campoli-Richards, Deborah M.; Goa, Karen L.,(1989) a review on the anti- bacterial activity of sultamcillintosilate, pharmacokinetic properties and therapeutic use, vol-37(1) Pp 54-67

31. A'K.I. Airede, I. Jalo ,H.D. Weerasinghe ,M. Bello, 'SegunAdeyemi (1996) presented a review on the neonatal use of sultamcillintosilate, journal of antimicrobial agents, vol- 22(1), Pp55-59.

32 .Changquin H., Wei L., Minzhi Y., Shaohong J, Chines Journal of antibiotics (1997)., vol-22 (2)Pp182-186.

33. El-Shanawani A.A .,ActaPoloniaepharmaceutica- Drug research(1998).,vol-55(1) Pp9-14.

34.Pneumologie I(2001) Internationa journal of Antimicrobial agents ; reported the use of Sultamcillin tosilate in the treatment of upper and lower respiratory tract infections. Vol-18(2)Pp199-209.

35.Pajchel G and Tyski S.,(2002) in the journal of Chromatography discussed about the determination of sultamcillin by micellarelectrokinetic capillary electrophoretic method for determination of Sultamicillin in Unasyn oral preparations like tablets and suspensions. Vol-53(3)Pp315-321

36.ZHangQing yuan .,(2004)journal of anti microbial chemotherapy reported the pharmacokinetics of Sultamcillin tosilate,vol-17(4)Pp529-533.

37. BhosleD,BharambeS,GairolaN,DhaneshwarSS,(2006)reported on Mutual prodrug concept;Fundamentals and Applications, Indian journal of Pharmaceutical sciences, vol- 28(2)Pp286-294.

38.H. J. Rogers,ID.Bradbrook,P J morrison,R.G Spector,(2009) in Journal of Antimicrobial therapy reported on the plasma level studies of sultamcillin, vol-11(2)Pp 124-130

39.Mustafa Polat, Mustafa Korkmaz, Radiation effects and Defects in solids; Incorporating plasma science and plasma technology et al.,(2009)vol-164(2)Pp90-100.

40.W Schutz.,(2009) reported on open randomized multi-centric study of sultamcillin, International journal of medical research,vol-21(1)Pp-41-45

41.V J Kumar, P B Gupta *et al*[24]., (2011) in journal of Pharmaceutica and bio medical analysis, vol-54(3)Pp-582-587.

42.www.ctshplc.com/pump-Icresources.com

43.www. Formuscil.co.il

44.www.hplcsys.gif

45.www. Agela.com/chemistry.nmsu.edu.

46.www.wikipedia.com, high performanc thin layer chromatography

43. www.structure.com